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THE DISTRIBUTION OF ERRORS FOR THE DETERMINATION OF PROTEIN, MOISTURE AND ASH IN FLOUR [1]

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Introduction

In the quantitative evaluation of constituents of materials by processes which involve chemical or physical change in those materials, recognition must be given to at least four general sources of variability which may be expected to affect the accuracy of the results. This total variability may be designated as the *error of analysis*, and its sources may be classified as errors of:

1. Sampling,
2. Chemical impurity,
3. Type and rate of chemical or physical change,
4. Measurement, attributable to apparatus and personal judgment.

While it is not the purpose of the present contribution to the problem of errors of chemical analysis to discuss in detail the origins of such variations, it would appear to be of advantage to focus attention upon contributary causes of error by presenting such a classification with a few brief comments in elaboration of the categories given.

It may be noted that lack of perfect homogeneity in the density of distribution of the constituents of a material being analyzed will inevitably introduce an error of sampling in the preparation of fractions for analysis. It should be clearly understood that the term homogeneity is used here, as generally elsewhere, in a relative sense. The ideal of *perfect* homogeneity may never be anticipated in any mixture of discrete particles. However, comparative homogeneity is readily attributable to a solution in comparison with a mixture of solids such as is represented by wheat flour, and the errors of sampling in the former may readily be considered negligible in comparison with those of the

[1] This paper was read as a subcommittee report of the Committee on Methods of Analyses.

latter. This source of error may be reduced only by increasing the degree of homogeneity of the material to be analyzed prior to the drawing of the sample, or by the use of samples for analysis of greater mass. The error is a purely statistical phenomenon in that the final result forms a true average of every series of smaller samples into which the chosen sample might conceivably be divided.

The second group of errors, due to impurity in the chemicals introduced in the analysis, needs no descriptive elaboration here. The category is intended to embrace only those impurities which would appear finally as an increment added to the constituent being determined. Of another character are those which influence, catalytically or otherwise, the rate or type of reaction with consequent influence on the final result; these impurities are classified in the next group. Neglecting the quite minor sampling errors attendant upon securing the necessary amounts of the chemicals employed, the error introduced by such impurities may be expected to be constant so long as the chemical supply is fixed. Hence it may be designated as a *systematic* error in contrast to the variable or *random* error of sampling.

Errors arising from differences in the rate or even type of chemical or physical change consummated in an analysis form the third category in the present scheme. They may be expected to have their origins chiefly in differences between units of physical equipment, or character of chemicals employed, which result in differences (as between analyses replicated in the same or different laboratories) in (*a*) the intensity of energy supplied to the particles entering change of state, (*b*) the precise type of reaction, and (*c*) the rate at which final products are removed from influence on the reaction rate. In the determination of nitrogen, by the Kjeldahl process or its many modifications, for example, the energy supply in digestion is commonly known to vary over a wide range from laboratory to laboratory, and even from burner to burner in the same laboratory when a constant line voltage or gas pressure is maintained. Again, the validity of a moisture determination is known to be a function, in part, of the method of removal of water vapor from wheat, and of the interval of heating and position in different ovens for flour samples. Impurities in chemicals may exert, catalytically or otherwise, sufficient influence on the type and final equilibrium of a reaction [2] to differentiate results from laboratories working independently.

It will be clear, then, that this third class covers, in some cases at least, a multitude of errors. It is maintained here as a unit solely to avoid an extensive multiplication of descriptive categories which would

[2] One is lead to wonder, sometimes, how many chemical reactions proceed as simply as the elementary equations would indicate.

vary largely according to the analysis being investigated. These errors, in their total effect, may be expected to be mainly of a systematic character, this statement obviously depending for its justification upon a degree of constancy in replication which may not be easy to maintain. Thus, random errors may be superimposed upon the systematic errors at one or more points in the analysis, even during replications in the same laboratory. The complexity of any undertaking to theoretically evaluate in detail the components of such a group of errors is surely apparent.

The fourth and last category of errors to be considered here concerns that complex aggregation of physical equipment of the laboratory and physiologic variability of the analyst employing such equipment, which, in a collective sense, may be designated as the mechanism of measurement. In practical work these two components are essentially bound one to the other since, except in the case of recording discrete numbers where there is no possibility of error, human judgment through visual impression is essential to the employment of any physical instrument. Most exacting calibration of a piece of apparatus by one individual might not be completely verified by another working independently. Errors in the calibration of apparatus will be cumulative from one instrument to another, the sum being large or small depending upon compensation of the individual errors and their magnitudes. Systematic errors of human judgment such as arise through an habitual error of parallax in reading an instrument, will be cumulative in the same sense as calibration errors. On the other hand, the random errors which so prominently characterize all human judgments, while they may be compensatory in individual combinations, will be additive in their general effect.

It is not a matter of concern at present to push theoretical considerations in any sense beyond the recognition of certain principles, which certainly seem to merit consideration in view of the practical results which follow in this paper. The statistical analysis of an extensive series of collaborative results such as is dealt with herein may only detect and measure the influence of the two general types of error, the random and the systematic. Vastly greater and more detailed work would be required to segregate such errors into their final components—a task whose profit would hardly be commensurate with the labor involved. It is worthy of note on the theoretical side, however, that if every source of error in an analysis be known and quantitatively evaluated, then the variance of the analyses would be a simple sum of the variances belonging to each source of error. Failure of the total error to agree with the sum of known contributing parts would prove

indisputable evidence that account had not been taken of all the individual sources of error.

In such routine analyses as those for protein, moisture, and ash in flour, errors of considerable magnitude occur. The critical and unprejudiced analyst will find, in exploring the possible causes, a measure of profit far exceeding whatever satisfaction arises through propounding explanations in particular cases for departure from some imagined standard of accuracy. There is in many places today an inadequate appreciation of the extent of error in chemical analyses. *This is a situation which has very naturally arisen because attention has been focussed not upon extensive replication of analyses, but upon securing a desired concordance of duplicate analyses.* The organization of systems of "check analyses," performed at reasonably frequent intervals among groups such as are provided by the local sections of the Association, with periodical employment of the same sample for analysis by all such groups, would provide a most desirable organization for the evaluation and elimination of systematic errors, also for the evaluation and reduction to a basic level of the random errors of analysis. The success of such an undertaking would depend not only on willingness to cooperate in performing analyses, but also on readiness to act in the control of errors by individual analysts once those errors have been established.

Historical

The significance of variation in the results of chemical analyses of considerable importance to the cereal industry and performed in a more or less routine manner by cereal chemists was first established by Treloar and Harris (1928). In illustrating the use of statistical techniques appropriate to testing the validity of analytical procedures, they studied certain relatively large series of data published in the annual reports of the Committee on Methods of Analysis of the American Association of Cereal Chemists, and demonstrated therein the existence of errors of a systematic as well as a random character. These errors, occurring in protein, moisture and ash reports from a large number of laboratories analyzing samples of wheat or flour, were of such a magnitude as to render the data of little value for the purposes for which they were collected.

In the following year (1928–9) the committee as above, under M. J. Blish as Chairman, sponsored a collaborative study of a critical nature under the direction of the writer to ascertain what degree of concordance might be reached within and between selected laboratories in replicated analyses for protein in 3 flours, one each of low, medium, and high protein content. The statistical analysis of these data

(Treloar, 1929) showed that protein determinations might be expected to vary over a range of approximately three tenths of one per cent when replicated within laboratories of high precision, and over twice that range as between laboratories, the latter increase being due to systematic errors characterizing the work of analysts. These ranges were based upon an assumption of normality of distribution of error which appeared to form a reasonable working hypothesis. These results showed that the error was far greater than that which might be expected on *a priori* theoretical considerations, and clearly indicated the fallacy of reporting protein analyses to hundredths of one per cent, as was all too commonly the case. The fairly general adoption since that time of the principle that protein analyses on wheat and flour should not be reported to smaller fractions than tenths of one per cent (unless the average of a large series is being given) rests largely on the foundation provided by that investigation.

Experimental

The present study aims to considerably widen the investigation of errors of analysis by extending the enquiry (1) to a large number of laboratories, and (2) to cover moisture and ash determinations as well as protein. As material for analysis a commercial wheat flour (bakers' patent) was selected.

The following results suffer the restriction that they arise from the study of a single flour sample. Since all flours do not digest nor ash with equal facility, and also errors in weighing out samples for analysis and draining solutions of the sample from measuring apparati may be expected to be proportional under a fixed technique to the quantity of the constituent being determined, the nature of this limitation cannot be ignored. However, it did not seem possible to organize a wide collaborative study of a large number of samples of flour. Accordingly a "typical" flour was selected and the project of comparing error distributions for different flours was left for subsequent investigation.

The flour was carefully mixed by stirring and sieving to reduce heterogeneity (and hence the sampling error) as much as possible, and was rapidly distributed among approximately 140 sample tins by two workers to maintain uniformity of moisture content. The tins were immediately closed and sealed with adhesive tape, and then sent to the chairmen of local sections for distribution to such laboratories as would be in the best position to include the tests in *routine* analytical work. In the Northwest Section, the sample formed one of the bimonthly "check tests" and accordingly all laboratories cooperating in that work received the flour for analysis. Chairmen of other sections distributed up to 15 samples within their respective groups.

The following excerpts from the letter of instructions forwarded to each collaborator with the sample for analysis indicate the form of request made:

"You are being asked to cooperate with the Methods Committee of the A. A. C. C. in a survey project for the determination of the degree of consistency that is characteristic of everyday routine work in replicated analyses for protein, moisture, and ash in flour. Complete honesty in reporting *every individual determination* is essential to the success of the project—the check is not on the analyst, but on the validity of the methods. You will receive one sample of flour (No. 172) and in analyzing same are requested to comply with the following suggestions as closely as is feasible without disturbing customary routine accuracy.

"Weigh four (4) portions of the flour for each analysis (protein, moisture, and ash) at the same time and with the usual degree of precision, with the minimum exposure of the flour sample to drying conditions before the weighing is completed.

"Include the samples *in pairs* in the regular run, concealing the identity of the pairs if possible. That is, run individuals 1 and 2 together under one number, and 3 and 4 somewhat later (the next day, if necessary) under a different number so that it genuinely appears to be a different sample.

"Use the official methods wherever your regular routine permits of a choice, and indicate the method used.

"Report results in the space provided in the following table *without modification*, i.e., to the degree of accuracy given by the final reading of the burette in the case of protein, and to two and three places of decimals for moisture and ash, respectively, if the *weighing technique* justifies same."

The objective in splitting the quadruplicate analyses into two separated pairs of concurrent duplicates was to test for systematic differentiation between individuals of a pair of analyses. The writer has striking evidence of the existence of such differentiation in an unusually large series of analyses for protein in two laboratories, the accuracy of whose work is of very great commercial importance. The existence of such a differentiation among laboratories in general would be a matter of considerable significance as it would demand a selection of either the first or the second analysis as being more accurate on the average than the mean of both.

It was desired that the results should reflect errors as they customarily occur, hence specifications concerning analysis which might lead to particular treatment of the sample were avoided. It is assumed in the following treatment of the results that the errors shown are representative of the analyses as they are performed in everyday routine. For the present, consideration will not be given to the methods of analysis reported as being employed by the collaborators.

Of the 133 sub-samples distributed, analyses were performed and reports rendered on 109. Of these, two series of protein determinations were not included in the calculations because of apparent mistakes, one in each series. They are footnoted in Table I. Three other reports had unfortunately to be entirely rejected because failure to follow the instructions given destroyed their value for the present purposes. In all, the work of 106 collaborators is being studied in this investigation, 99 reporting on protein, 96 on moisture and 95 on ash.

Upon receipt of the results from each collaborator, a number was awarded the report and statistical reduction of the data proceeded with. The mean and standard deviation of each set of four analyses was first obtained, the individual results being then expressed as "errors" or deviations from the mean. In this form the reports are summarized as Tables I, II, and III.

Results

(1) *The Random Error of Analysis*

In the absence of differentiation between the first and second determinations of consecutive pairs, and between the results from a first pair and a second pair in the same laboratory, then the discrepancies between the four determinations and their average will provide a measure of the individual random errors of analysts of each collaborator. A quantitative measure of the extent of such error will be provided by the standard deviation of the four analyses for each worker, since $\sigma_{(x-\bar{x})} = \sigma_x$. However, such standard deviations will be less than the true values based on a large series of replicates, since the means from which they are measured are only approximations to the true mean for the sample. Theoretically, the mean of an infinite series of replicates in the same laboratory would be free from random error, and it is from such a mean that the error of individual analyses should be measured.

Let X be the true value of the constituent, and x_1, x_2, x_3 and x_4 be four analytical determinations estimating X. Then the true errors of analysis are $(x_1 - X)$, $(x_2 - X)$, $(x_3 - X)$ and $(x_4 - X)$. Since X is unknown, the mean, $\bar{x}$, of the four analyses must be used in its place. Let e be the error in estimating X from $\bar{x}$. Then $e = (\bar{x} - X)$, and will be sometimes positive and sometimes negative. Now $X = (\bar{x} - e)$ and $(x - X) = (x - \bar{x}) + e$. Therefore $\sigma_{(x-X)} = \sigma_{(x-\bar{x})+e}$, a quantity greater than $\sigma_{(x-\bar{x})} = \sigma_x$. Accordingly it will be necessary to correct the standard deviations of the quadruplicate analyses in the direction of increase to take care of these errors in the means.

Firstly, it is necessary to test for differentiation in and between the pairs of analyses as performed in this collaborative experiment. This has been accomplished by calculating the individual differences between analyses and determining the average of each comparison for the entire series of collaborators. In the absence of systematic differentiation, such averages should be equal to zero. The average differences with standard errors and ratios are presented in Table IV. The means in every case are of very small magnitude. Also the ratios of these means to their respective standard errors are less than 2 in

TABLE I

DATA FOR PROTEIN IN FLOUR SAMPLE 172

Coll. number	Analysis number				Mean	St. Dev. (σ_4)	Errors ($\times 10^2$)			
	(1)	(2)	(3)	(4)			(1)	(2)	(3)	(4)
3	14.50	14.60	14.60	14.63	14.58	.0492	− 8	+ 2	+ 2	+ 5
4	14.52	14.50	14.48	14.65	14.54	.0665	− 2	− 4	− 6	+11
5	14.45	14.50	14.45	14.50	14.48	.0250	− 3	+ 3	− 3	+ 3
6	14.64	14.60	14.60	14.66	14.63	.0260	+ 2	− 3	− 3	+ 4
8	14.61	14.61	14.69	14.61	14.63	.0346	− 2	− 2	+ 6	− 2
10	14.60	14.64	14.64	14.64	14.63	.0173	− 3	+ 1	+ 1	+ 1
11	14.45	14.35	14.50	14.50	14.45	.0612	0	−10	+ 5	+ 5
12	14.70	14.65	14.70	14.70	14.69	.0217	+ 1	− 4	+ 1	+ 1
13	14.50	14.50	14.50	14.55	14.51	.0217	− 1	− 1	− 1	+ 4
15	14.40	14.70	14.65	14.67	14.61	.1197	−21	+10	+ 5	+ 7
16	14.60	14.60	14.60	14.68	14.62	.0346	− 2	− 2	− 2	+ 6
17	14.24	14.24	14.40	14.40	14.32	.0800	− 8	− 8	+ 8	+ 8
18	12.19	12.20	12.18	12.20	12.19	.0083	− 0	+ 1	− 1	+ 1
19	14.72	14.72	14.80	14.80	14.76	.0400	− 4	− 4	+ 4	+ 4
20	14.76	14.62	14.71	14.71	14.70	.0505	+ 6	− 8	+ 1	+ 1
21	14.76	14.76	14.80	14.78	14.78	.0166	− 2	− 2	+ 3	+ 1
22	14.68	14.52	14.68	14.68	14.64	.0693	+ 4	−12	+ 4	+ 4
24	14.72	14.62	14.84	14.77	14.74	.0801	− 2	−12	+10	+ 3
25	15.16	15.08	15.16	15.32	15.18	.0872	− 2	−10	− 2	+14
26	14.64	14.48	14.56	14.48	14.54	.0663	+10	− 6	+ 2	− 6
27	14.70	14.70	14.70	14.70	14.70	.0000	0	0	0	0
29	14.55	14.64	14.60	14.60	14.60	.0319	− 5	+ 4	+ 0	+ 0
30[1]	14.54	14.39	14.54	16.62						
31	14.44	14.48	14.52	14.64	14.52	.0748	− 8	− 4	0	+12
32	14.63	14.76	14.75	14.76	14.73	.0550	−10	+ 4	+ 3	+ 4
33	14.50	14.60	14.60	14.52	14.56	.0456	− 6	+ 5	+ 5	− 4
34	14.60	14.70	14.50	14.40	14.55	.1118	+ 5	+15	− 5	−15
36	14.44	14.52	14.52	14.60	14.52	.0566	− 8	0	0	+ 8
39	14.60	14.55	14.64	14.60	14.60	.0319	+ 0	− 5	+ 4	+ 0
40[1]	14.63	14.65	15.50	14.85						
43	14.56	14.60	14.64	14.60	14.60	.0283	− 4	0	+ 4	0
44	14.70	14.70	14.70	14.70	14.70	.0000	0	0	0	0
45	14.35	14.35	14.40	14.25	14.34	.0545	+ 1	+ 1	+ 6	− 9
47	14.60	14.52	14.52	14.52	14.54	.0346	+ 6	− 2	− 2	− 2
48	14.68	14.76	14.76	14.68	14.72	.0400	− 4	+ 4	+ 4	− 4
49	14.80	14.70	14.70	14.65	14.71	.0545	+ 9	− 1	− 1	− 6
50	14.62	14.66	14.70	14.74	14.68	.0447	− 6	− 2	+ 2	+ 6
56	11.60	11.60	11.65	11.60	11.61	.0217	− 1	− 1	+ 4	− 1
57	14.69	14.73	14.70	14.69	14.70	.0164	− 1	+ 3	− 0	− 1
59	14.76	14.82	14.82	14.93	14.83	.0614	− 7	− 1	− 1	+10
60	14.84	14.72	14.84	14.76	14.79	.0520	+ 5	− 7	+ 5	− 3
61	14.70	14.70	14.75	14.70	14.71	.0217	− 1	− 1	+ 4	− 1
62	14.66	14.70	14.70	14.70	14.69	.0173	− 3	+ 1	+ 1	+ 1
65	14.60	14.62	14.68	14.70	14.65	.0412	− 5	− 3	+ 3	+ 5
66	14.60	14.55	14.60	14.60	14.59	.0217	+ 1	− 4	+ 1	+ 1
68	14.60	14.60	14.60	14.65	14.61	.0217	− 1	− 1	− 1	+ 4
69	14.50	14.55	14.55	14.55	14.54	.0217	− 4	+ 1	+ 1	+ 1
70	14.80	14.70	14.70	14.70	14.73	.0433	+ 8	− 3	− 3	− 3
71	14.40	14.60	14.55	14.65	14.55	.0935	−15	+ 5	0	+10
72	14.64	14.72	14.72	14.64	14.68	.0400	− 4	+ 4	+ 4	− 4
73	14.70	14.80	14.70	14.70	14.73	.0433	− 3	+ 8	− 3	− 3

TABLE I (*Cont'd*)

Coll. number	Analysis number (1)	(2)	(3)	(4)	Mean	St. Dev. (σ_4)	Errors ($\times 10^2$) (1)	(2)	(3)	(4)
75	14.65	14.55	14.80	14.85	14.71	.1192	− 6	−16	+ 9	+14
76	14.80	14.80	14.75	14.70	14.76	.0415	+ 4	+ 4	− 1	− 6
78	14.70	14.70	14.70	14.70	14.70	.0000	0	0	0	0
80	14.60	14.60	14.50	14.60	14.58	.0433	+ 3	+ 3	− 8	+ 3
81	14.60	14.55	14.60	14.50	14.56	.0415	+ 4	− 1	+ 4	− 6
82	14.70	14.68	14.68	14.68	14.69	.0087	+ 2	− 1	− 1	− 1
83	14.54	14.60	14.65	14.58	14.59	.0396	− 5	+ 1	+ 6	− 1
84	14.66	14.64	14.64	14.54	14.62	.0469	+ 4	+ 2	+ 2	− 8
85	14.90	14.80	14.80	14.80	14.83	.0433	+ 8	− 3	− 3	− 3
88	14.70	14.60	14.60	14.60	14.63	.0433	+ 8	− 3	− 3	− 3
90	14.70	14.80	14.60	14.70	14.70	.0707	0	+10	−10	0
91	14.45	14.57	14.61	14.61	14.56	.0656	−11	+ 1	+ 5	+ 5
92	14.60	14.60	14.55	14.55	14.58	.0250	+ 3	+ 3	− 3	− 3
93	14.69	14.70	14.76	14.71	14.72	.0269	− 3	− 2	+ 5	− 1
94	14.70	14.60	14.65	14.80	14.69	.0740	+ 1	− 9	− 4	+11
95	14.55	14·60	14.50	14.45	14.53	.0559	+ 3	+ 8	− 3	− 8
96	14.68	14.76	14.76	14.80	14.75	.0436	− 7	+ 1	+ 1	+ 5
98	14.50	14.60	14.55	14.60	14.56	.0415	− 6	+ 4	− 1	+ 4
99	14.60	14.60	14.60	14.70	14.63	.0433	− 3	− 3	− 3	+ 8
100	14.55	14.60	14.60	14.60	14.59	.0217	− 4	+ 1	+ 1	+ 1
101	14.77	14.69	14.58	14.62	14.67	.0723	+11	+ 3	− 9	− 5
102	14.70	14.70	14.70	14.75	14.71	.0217	− 1	− 1	− 1	+ 4
103	14.68	14.68	14.68	14.76	14.70	.0346	− 2	− 2	− 2	+ 6
104	14.75	14.75	14.68	14.68	14.72	.0350	+ 4	+ 4	− 4	− 4
105	14.24	14.28	14.36	14.44	14.33	.0768	− 9	− 5	+ 3	+11
106	14.68	14.64	14.64	14.60	14.64	.0283	+ 4	0	0	− 4
107	14.64	14.62	14.58	14.62	14.62	.0218	+ 3	+ 1	− 4	+ 1
109	14.68	14.62	14.56	14.58	14.61	.0458	+ 7	+ 1	− 5	− 3
110	14.62	14.70	14.70	14.65	14.67	.0342	− 5	+ 3	+ 3	− 2
111	14.43	14.43	14.52	14.61	14.50	.0746	− 7	− 7	+ 2	+11
112	14.72	14.68	14.72	14.72	14.71	.0173	+ 1	− 3	+ 1	+ 1
113	14.60	14.64	14.60	14.68	14.63	.0332	− 3	+ 1	− 3	+ 5
114	14.55	14.45	14.45	14.45	14.48	.0433	+ 8	− 3	− 3	− 3
115	14.65	14.63	14.53	14.63	14.61	.0469	+ 4	+ 2	− 8	+ 2
116	14.68	14.74	14.68	14.63	14.68	.0390	− 0	+ 6	− 0	− 5
117	14.42	14.45	14.49	14.45	14.45	.0249	− 3	− 0	+ 4	− 0
118	14.56	14.52	14.60	14.56	14.56	.0283	0	− 4	+ 4	0
119	14.56	14.60	14.64	14.56	14.59	.0332	− 3	+ 1	+ 5	− 3
120	14.70	14.70	14.75	14.79	14.74	.0377	− 4	− 4	+ 2	+ 6
121	14.65	14.62	14.70	14.60	14.64	.0377	+ 1	− 2	+ 6	− 4
122	14.24	14.24	14.32	14.24	14.26	.0346	− 2	− 2	+ 6	− 2
124	14.60	14.65	14.60	14.60	14.61	.0217	− 1	+ 4	− 1	− 1
125	14.36	14.36	14.31	14.36	14.35	.0217	+ 1	+ 1	− 4	+ 1
126	14.53	14.59	14.65	14.65	14.61	.0497	− 8	− 2	+ 5	+ 5
127	14.70	14.50	14.55	14.55	14.58	.0750	+13	− 8	− 3	− 3
128	14.36	14.28	14.36	14.44	14.36	.0566	0	− 8	0	+ 8
129	14.50	14.50	14.55	14.61	14.54	.0453	− 4	− 4	+ 1	+ 7
130	14.68	14.68	14.72	14.70	14.70	.0166	− 2	− 2	+ 3	+ 1
131	14.60	14.52	14.48	14.52	14.53	.0436	+ 7	− 1	− 5	− 1
133	14.64	14.64	14.68	14.68	14.66	.0200	− 2	− 2	+ 2	+ 2

[1] Dropped from calculations because of an apparent mistake in analysis.

TABLE II

DATA FOR MOISTURE IN FLOUR SAMPLE 172

Coll. number	Analysis number				Mean	St. Dev. (σ_4)	Errors ($\times 10^2$)			
	(1)	(2)	(3)	(4)			(1)	(2)	(3)	(4)
3	13.22	13.19	13.19	13.15	13.19	.0249	+ 3	+ 0	+ 0	− 4
4	13.23	13.25	13.29	13.24	13.25	.0228	− 2	− 0	+ 4	− 1
5	13.30	13.30	13.30	13.30	13.30	.0000	0	0	0	0
6	13.20	13.30	13.10	13.20	13.20	.0707	0	+10	−10	0
8	13.30	13.28	13.30	13.27	13.29	.0130	+ 1	− 1	+ 1	− 2
10	13.20	13.15	13.00	13.00	13.09	.0893	+11	+ 6	− 9	− 9
11	12.96	13.03	13.05	13.15	13.05	.0680	− 9	− 2	+ 0	+10
13	13.10	13.10	13.15	13.20	13.14	.0415	− 4	− 4	+ 1	+ 6
15	13.24	13.20	13.08	12.98	13.13	.1023	+12	+ 8	− 5	−15
16	13.35	13.18	13.25	13.23	13.25	.0618	+10	− 7	− 0	− 2
17	12.70	12.70	12.70	12.70	12.70	.0000	0	0	0	0
18	15.04	14.98	15.08	15.10	15.05	.0458	− 1	− 7	+ 3	+ 5
19	13.25	13.20	13.10	13.10	13.16	.0650	+ 9	+ 4	− 6	− 6
20	13.30	13.30	13.30	13.40	13.33	.0433	− 3	− 3	− 3	+ 8
21	13.20	13.25	13.25	13.30	13.25	.0354	− 5	0	0	+ 5
22	13.03	13.08	13.05	13.08	13.06	.0212	− 3	+ 2	− 1	+ 2
24	13.12	13.20	13.14	13.18	13.16	.0316	− 4	+ 4	− 2	+ 2
25	12.92	12.94	12.79	12.70	12.84	.0981	+ 8	+10	− 5	−14
26	12.88	12.68	12.62	12.87	12.76	.1145	+12	− 8	−14	+11
27	13.30	13.30	13.26	13.26	13.28	.0200	+ 2	+ 2	− 2	− 2
29	13.33	13.31	13.19	13.23	13.27	.0572	+ 7	+ 5	− 8	− 4
30	13.12	13.09	13.14	13.11	13.12	.0180	+ 1	− 3	+ 3	− 1
31	13.10	13.10	13.00	12.90	13.03	.0829	+ 8	+ 8	− 3	−13
32	12.76	12.88	12.92	12.93	12.87	.0676	−11	+ 1	+ 5	+ 6
33	13.26	13.30	13.30	13.24	13.28	.0260	− 2	+ 3	+ 3	− 4
34	12.90	12.90	13.12	13.10	13.01	.1052	−11	−11	+12	+10
36	12.90	12.95	13.10	13.00	12.99	.0740	− 9	− 4	+11	+ 1
39	13.40	13.35	13.30	13.38	13.36	.0377	+ 4	− 1	− 6	+ 2
40	13.13	13.15	13.14	13.14	13.14	.0071	− 1	+ 1	0	0
43	13.26	13.20	13.23	13.28	13.24	.0303	+ 2	− 4	− 1	+ 4
44	13.41	13.35	13.35	13.25	13.34	.0574	+ 7	+ 1	+ 1	− 9
45	13.45	13.50	13.50	13.50	13.49	.0217	− 4	+ 1	+ 1	+ 1
47	13.03	13.04	13.10	13.05	13.06	.0269	− 3	− 2	+ 5	− 1
48	13.00	13.00	13.00	13.05	13.01	.0217	− 1	− 1	− 1	+ 4
49	13.20	13.20	13.10	13.20	13.18	.0433	+ 3	+ 3	− 8	+ 3
50	13.10	13.00	13.10	13.00	13.05	.0500	+ 5	− 5	+ 5	− 5
59	13.50	13.25	13.50	13.25	13.38	.1250	+13	−13	+13	−13
60	13.30	13.30	13.28	13.30	13.30	.0087	+ 1	+ 1	− 2	+ 1
62	13.20	13.10	13.00	13.10	13.10	.0707	+10	0	−10	0
67	13.30	13.35	13.37	13.35	13.34	.0259	− 4	+ 1	+ 3	+ 1
69	13.45	13.50	13.40	13.40	13.44	.0415	+ 1	+ 6	− 4	− 4
70	13.27	13.30	13.27	13.23	13.27	.0249	+ 0	+ 3	+ 0	− 4
71	13.34	13.26	13.41	13.42	13.36	.0642	− 2	−10	+ 5	+ 6
72	13.30	13.30	13.20	13.20	13.25	.0500	+ 5	+ 5	− 5	− 5
73	13.30	13.36	13.10	13.13	13.22	.1101	+ 8	+14	−12	− 9
74	13.00	13.10	13.00	13.20	13.08	.0829	− 8	+ 3	− 8	+13
75	13.18	13.30	13.32	13.29	13.27	.0545	− 9	+ 3	+ 5	+ 2
76	13.43	13.41	13.43	13.40	13.42	.0130	+ 1	− 1	+ 1	− 2

TABLE II (*Cont'd*)

Coll. number	Analysis number (1)	(2)	(3)	(4)	Mean	St. Dev. (σ_4)	Errors ($\times 10^2$) (1)	(2)	(3)	(4)
78	13.34	13.33	13.30	13.30	13.32	.0179	+ 2	+ 1	− 2	− 2
80	12.98	12.99	12.87	12.88	12.93	.0552	+ 5	+ 6	− 6	− 5
81	13.31	13.28	13.29	13.30	13.30	.0112	+ 2	− 2	− 1	+ 1
83	13.30	13.10	13.30	13.20	13.23	.0829	+ 8	−13	+ 8	− 3
84	13.10	13.10	13.30	13.30	13.20	.1000	−10	−10	+10	+10
85	13.10	13.10	13.15	13.10	13.11	.0217	− 1	− 1	+ 4	− 1
87	13.30	13.25	13.30	13.30	13.29	.0217	+ 1	− 4	+ 1	+ 1
88	13.21	13.22	13.22	13.33	13.25	.0492	− 4	− 3	− 3	+ 9
89	13.20	13.20	13.20	13.20	13.20	.0000	0	0	0	0
90	13.00	13.02	12.85	12.86	12.93	.0779	+ 7	+ 9	− 8	− 7
91	13.20	13.20	13.00	13.00	13.10	.1000	+10	+10	−10	−10
92	13.30	13.20	13.10	13.10	13.18	.0829	+13	+ 3	− 8	− 8
94	13.20	13.20	13.10	13.10	13.15	.0500	+ 5	+ 5	− 5	− 5
95	12.97	12.97	12.90	12.88	12.93	.0406	+ 4	+ 4	− 3	− 5
96	12.71	12.68	12.77	12.83	12.75	.0576	− 4	− 7	+ 2	+ 8
98	13.23	13.21	13.26	13.26	13.24	.0212	− 1	− 3	+ 2	+ 2
99	12.90	12.90	13.00	13.10	12.98	.0829	− 8	− 8	+ 3	+13
100	12.95	12.90	13.00	13.00	12.96	.0415	− 1	− 6	+ 4	+ 4
101	12.84	12.78	13.07	12.89	12.90	.1083	− 6	−12	+18	− 1
102	13.18	13.15	13.15	13.10	13.15	.0287	+ 4	+ 1	+ 1	− 5
103	12.82	12.87	12.74	12.74	12.79	.0554	+ 3	+ 8	− 5	− 5
104	13.35	13.33	13.28	13.31	13.32	.0259	+ 3	+ 1	− 4	− 1
105	13.10	13.20	13.20	13.20	13.18	.0433	− 8	+ 3	+ 3	+ 3
106	13.30	13.30	13.36	13.36	13.33	.0300	− 3	− 3	+ 3	+ 3
107	12.92	12.93	13.00	13.03	12.97	.0464	− 5	− 4	+ 3	+ 6
109	13.15	13.20	13.15	13.15	13.16	.0217	− 1	+ 4	− 1	− 1
110	13.29	13.19	13.25	13.11	13.21	.0656	+ 8	− 2	+ 4	−10
111	13.12	13.10	13.01	12.99	13.06	.0559	+ 7	+ 5	− 5	− 7
112	13.35	13.40	13.50	13.40	13.41	.0545	− 6	− 1	+ 9	− 1
113	12.80	13.20	13.10	13.30	13.10	.1871	−30	+10	0	+20
114	13.30	13.25	13.15	13.20	13.23	.0559	+ 8	+ 3	− 8	− 3
115	13.33	13.30	13.32	13.37	13.33	.0255	0	− 3	− 1	+ 4
116	13.01	13.01	13.07	13.10	13.05	.0390	− 4	− 4	+ 2	+ 5
117	13.30	13.30	13.20	13.27	13.27	.0409	+ 3	+ 3	− 7	+ 0
118	13.40	13.35	13.35	13.35	13.36	.0217	+ 4	− 1	− 1	− 1
119	13.30	13.20	13.20	13.20	13.23	.0433	+ 8	− 3	− 3	− 3
120	13.18	13.01	13.02	12.89	13.03	.1031	+16	− 2	− 1	−14
121	13.24	13.19	13.18	13.22	13.21	.0239	+ 3	− 2	− 3	+ 1
122	13.00	13.00	12.80	12.80	12.90	.1000	+10	+10	−10	−10
124	12.82	12.83	12.93	12.84	12.86	.0439	− 4	− 3	+ 8	− 2
125	13.00	12.95	13.00	13.00	12.99	.0217	+ 1	− 4	+ 1	+ 1
126	13.24	13.06	13.08	13.13	13.13	.0698	+11	− 7	− 5	+ 0
127	13.15	13.09	13.21	13.10	13.14	.0476	+ 1	− 5	+ 7	− 4
128	12.96	13.00	12.88	12.80	12.91	.0768	+ 5	+ 9	− 3	−11
129	12.35	12.45	12.40	12.42	12.41	.0364	− 6	+ 5	− 1	+ 2
130	13.36	13.45	13.28	13.29	13.35	.0680	+ 2	+11	− 7	− 6
131	13.09	13.07	13.01	12.97	13.04	.0477	+ 6	+ 4	− 3	− 7
133	13.32	13.34	13.24	13.22	13.28	.0510	+ 4	+ 6	− 4	− 6

TABLE III

DATA FOR ASH IN FLOUR SAMPLE 172

Coll. number	Analysis number (1)	(2)	(3)	(4)	Mean	St. Dev. (σ_4)	Errors ($\times 10^3$) (1)	(2)	(3)	(4)
3	0.503	0.496	0.503	0.503	0.501	.0030	+ 2	− 5	+ 2	+ 2
4	.490	.483	.487	.480	.485	.0039	+ 5	− 2	+ 2	− 5
5	.487	.487	.493	.490	.489	.0024	− 2	− 2	+ 4	+ 1
6	.492	.494	.496	.496	.495	.0017	− 3	− 1	+ 2	+ 2
8	.490	.490	.490	.490	.490	.0000	0	0	0	0
10	.500	.500	.500	.500	.500	.0000	0	0	0	0
11	.497	.500	.500	.503	.500	.0022	− 3	0	0	+ 3
13	.505	.500	.505	.500	.503	.0026	+ 3	− 3	+ 3	− 3
15	.484	.496	.486	.496	.491	.0056	− 7	+ 6	− 5	+ 6
16	.496	.496	.500	.494	.497	.0022	− 1	− 1	+ 4	− 3
17	.487	.487	.490	.490	.489	.0015	− 2	− 2	+ 2	+ 2
18	.492	.494	.480	.482	.487	.0061	+ 5	+ 7	− 7	− 5
19	.476	.476	.480	.476	.477	.0017	− 1	− 1	+ 3	− 1
20	.486	.492	.492	.494	.491	.0030	− 5	+ 1	+ 1	+ 3
21	.474	.476	.478	.480	.477	.0022	− 3	− 1	+ 1	+ 3
22	.484	.474	.476	.474	.477	.0041	+ 7	− 3	− 1	− 3
24	.510	.500	.510	.500	.505	.0050	+ 5	− 5	+ 5	− 5
25	.462	.466	.470	.470	.467	.0033	− 5	− 1	+ 3	+ 3
26	.490	.490	.500	.510	.498	.0083	− 8	− 8	+ 3	+13
27	.493	.494	.496	.496	.495	.0010	− 2	− 1	+ 1	+ 1
29	.485	.485	.495	.485	.488	.0044	− 3	− 3	+ 8	− 3
30	.500	.500	.493	.500	.498	.0028	+ 2	+ 2	− 5	+ 2
31	.488	.496	.480	.480	.486	.0066	+ 2	+10	− 6	− 6
32	.493	.490	.487	.493	.491	.0024	+ 2	− 1	− 4	+ 2
33	.486	.492	.488	.486	.488	.0024	− 2	+ 4	0	− 2
34	.505	.505	.500	.490	.500	.0062	+ 5	+ 5	0	−10
36	.490	.506	.500	.490	.497	.0069	− 7	+10	+ 4	− 7
39	.445	.445	.438	.440	.442	.0031	+ 3	+ 3	− 4	− 2
40	.507	.520	.510	.507	.511	.0054	− 4	+ 9	− 1	− 4
43	.484	.488	.482	.484	.485	.0022	− 1	+ 4	− 3	− 1
44	.488	.494	.492	.492	.492	.0022	− 4	+ 3	+ 1	+ 1
45	.490	.485	.490	.490	.489	.0020	+ 1	− 4	+ 1	+ 1
47	.500	.497	.497	.497	.498	.0014	+ 2	− 1	− 1	− 1
48	.500	.500	.493	.500	.498	.0030	+ 2	+ 2	− 5	+ 2
49	.495	.490	.500	.495	.495	.0036	0	− 5	+ 5	0
50	.482	.488	.502	.504	.494	.0093	−12	− 6	+ 8	+10
59	.500	.496	.500	.506	.501	.0036	− 1	− 5	− 1	+ 6
60	.490	.486	.490	.490	.489	.0017	+ 1	− 3	+ 1	+ 1
62	.527	.520	.523	.527	.524	.0030	+ 3	− 4	− 1	+ 3
67	.490	.496	.496	.500	.496	.0036	− 6	+ 1	+ 1	+ 5
69	.496	.494	.506	.506	.501	.0056	− 5	− 7	+ 6	+ 6
70	.508	.508	.520	.508	.511	.0052	− 3	− 3	+ 9	− 3
71	.500	.497	.500	.490	.497	.0040	+ 3	+ 0	+ 3	− 7
72	.500	.500	.494	.496	.498	.0026	+ 3	+ 3	− 4	− 2
73	.483	.485	.483	.483	.484	.0010	− 1	+ 2	− 1	− 1
74	.482	.484	.484	.486	.484	.0014	− 2	0	0	+ 2

TABLE III (*Cont'd*)

Coll. number	Analysis number				Mean	St. Dev. (σ_4)	Errors ($\times 10^3$)			
	(1)	(2)	(3)	(4)			(1)	(2)	(3)	(4)
75	.490	.490	.484	.484	.487	.0030	+ 3	+ 3	− 3	− 3
76	.500	.502	.496	.496	.499	.0026	+ 2	+ 4	− 3	− 3
78	.482	.484	.486	.486	.485	.0017	− 3	− 1	+ 2	+ 2
80	.505	.500	.500	.510	.504	.0041	+ 1	− 4	− 4	+ 6
81	.478	.478	.478	.482	.479	.0017	− 1	− 1	− 1	+ 3
84	.500	.495	.485	.490	.493	.0057	+ 8	+ 3	− 8	− 3
85	.500	.493	.480	.480	.488	.0086	+12	+ 5	− 8	− 8
87	.470	.470	.475	.470	.471	.0020	− 1	− 1	+ 4	− 1
88	.510	.495	.500	.490	.499	.0073	+11	− 4	+ 1	− 9
89	.490	.490	.490	.490	.490	.0000	0	0	0	0
90	.520	.530	.500	.510	.515	.0112	+ 5	+15	−15	− 5
91	.492	.492	.485	.485	.489	.0036	+ 4	+ 4	− 4	− 4
92	.500	.505	.490	.485	.495	.0079	+ 5	+10	− 5	−10
94	.500	.500	.490	.490	.495	.0050	+ 5	+ 5	− 5	− 5
95	.460	.450	.460	.460	.458	.0044	+ 3	− 8	+ 3	+ 3
96	.480	.480	.490	.490	.485	.0050	− 5	− 5	+ 5	+ 5
98	.483	.487	.490	.490	.488	.0030	− 5	− 1	+ 3	+ 3
99	.480	.480	.490	.480	.483	.0044	− 3	− 3	+ 8	− 3
100	.485	.485	.490	.495	.489	.0041	− 4	− 4	+ 1	+ 6
101	.473	.480	.478	.480	.478	.0028	− 5	+ 2	+ 0	+ 2
102	.490	.493	.490	.493	.492	.0017	− 2	+ 2	− 2	+ 2
103	.484	.476	.486	.486	.483	.0041	+ 1	− 7	+ 3	+ 3
104	.497	.497	.497	.493	.496	.0017	+ 1	+ 1	+ 1	− 3
105	.497	.500	.500	.497	.499	.0017	− 2	+ 2	+ 2	− 2
106	.490	.492	.490	.488	.490	.0014	0	+ 2	0	− 2
107	.490	.490	.487	.490	.489	.0010	+ 1	+ 1	− 2	+ 1
109	.490	.493	.497	.493	.493	.0024	− 3	− 0	+ 4	− 0
110	.490	.490	.486	.486	.488	.0020	+ 2	+ 2	− 2	− 2
111	.498	.500	.500	.502	.500	.0014	− 2	0	0	+ 2
112	.497	.497	.497	.497	.497	.0000	0	0	0	0
113	.473	.477	.467	.467	.471	.0042	+ 2	+ 6	− 4	− 4
114	.500	.500	.500	.510	.503	.0044	− 3	− 3	− 3	+ 8
115	.492	.492	.498	.502	.496	.0042	− 4	− 4	+ 2	+ 6
116	.500	.506	.498	.504	.502	.0032	− 2	+ 4	− 4	+ 2
117	.490	.487	.490	.493	.490	.0022	0	− 3	0	+ 3
118	.490	.487	.490	.490	.489	.0010	+ 1	− 2	+ 1	+ 1
119	.492	.490	.492	.488	.491	.0017	+ 2	− 1	+ 2	− 3
120	.477	.467	.483	.480	.477	.0060	+ 0	−10	+ 6	+ 3
121	.482	.496	.488	.490	.489	.0050	− 7	+ 7	− 1	+ 1
122	.480	.482	.488	.485	.484	.0030	− 4	− 2	+ 4	+ 1
124	.490	.490	.490	.490	.490	.0000	0	0	0	0
125	.502	.500	.505	.495	.501	.0037	+ 2	− 1	+ 5	− 6
126	.507	.497	.497	.490	.498	.0061	+ 9	− 1	− 1	− 8
127	.494	.494	.496	.498	.496	.0017	− 2	− 2	+ 1	+ 3
128	.520	.510	.520	.515	.516	.0041	+ 4	− 6	+ 4	− 1
129	.500	.500	.500	.503	.501	.0010	− 1	− 1	− 1	+ 2
130	.490	.493	.497	.500	.495	.0039	− 5	− 2	+ 2	+ 5
131	.496	.494	.496	.500	.497	.0022	− 1	− 3	− 1	+ 4
133	.508	.510	.503	.506	.507	.0024	+ 1	+ 3	− 4	− 1

practically every instance. They are, therefore, not significantly different from zero in these cases. The two exceptions occur in the comparison of analyses for protein run on different days. Here the ratios are somewhat greater than 2, and suggest systematic errors differentiating one day's work from the next. However, in these cases the actual mean difference of 0.02% approximately may be neglected as far as its influence in the present study is concerned. Thus, it may be considered that the differences between individual analyses of each group of four are attributable on the whole to random errors.

TABLE IV

MEAN DISCREPANCIES BETWEEN ANALYSES (1), (2), (3), AND (4) FOR PROTEIN, MOISTURE AND ASH, WITH THEIR STANDARD ERRORS AND RATIOS

Analysis	Comparison within pairs		Comparison between pairs	
	(2)−(1)	(4)−(3)	(3)−(1)	(4)−(2)
Protein	.0013±.0077 (0.17)[1]	.0064±.0063 (1.02)	.0159±.0078 (2.04)	.0209±.0086 (2.43)
Moisture	−.0085±.0082 (1.05)	−.0018±.0074 (0.24)	−.0179±.0108 (1.66)	−.0111±.0101 (1.10)
Ash ($\times 10^4$)	0.84±5.43 (0.15)	−1.79±5.03 (0.36)	2.53±6.73 (0.38)	−0.11±7.47 (0.01)

[1] Ratio of the mean to its standard error.

Proceeding now to a study of the magnitudes of the random errors that characterize analyses for protein, moisture, and ash within laboratories, consideration will be given first to the average error of all laboratories combined, to be followed by a study of possible differences between laboratories in the extent of the error characterizing their work.

The average standard deviations of quadruplicates for all laboratories for the three analyses are given in the fourth column (headed σ_4) of Table V. It has been pointed out above that these values are too

TABLE V

THE AVERAGE MAGNITUDES OF RANDOM ERRORS

Analysis	No. of Coll.	Mean	$\bar{\sigma}_4$[1]	σ_∞[2]	Range[3]	C. V.
Protein	99	14.5660%	0.0427%	0.0535%	0.28%	.37%
Moisture	96	13.1619%	0.0513%	0.0642%	0.33%	.49%
Ash	95	0.4919%	0.0034%	0.0042%	0.022%	.85%

[1] Mean standard deviation of quadruplicates.
[2] Corrected standard deviation for infinite replication.
[3] Central 99% assuming a "normal" distribution of errors.

small as applied to infinite replication. Dividing by the factor of .7979 given by Pearson (1915) to correct the average standard deviation of samples of 4 to that of the population from which the samples are drawn, the values designated as σ_∞ are obtained. By multiplying

these latter values by 5.2 (approx., 5.1516$\cdots$ exactly) the ranges of the central 99% of the distributions of error are secured, assuming those distributions to be "normal." These latter values are of considerable interest, and their importance will be discussed fully later in this paper.

It is of interest here to note that, since the protein and moisture contents of the flour sample are reasonably comparable (14.6% and 13.2%, respectively) it is possible to compare the accuracy of these determinations directly in terms of their standard deviations. Thus it will be seen that the random error in the moisture determination in this large series of laboratories is approximately 20% greater than that of the protein determination. If it be hypothecated that errors become comparable when considered as a proportion of the magnitude of the constituent itself, all three determinations might be compared for their relative validity in terms of their coefficients of variation. These are given in the last column of Table V. On such a basis it would follow that the moisture determination is subject to about 30% more error than the protein determination, that for ash being of the order of double the error of these two analyses. However, some, if not many, of the components of the total error in a determination are in no way related to the magnitude of the constituent being determined. The writer feels, therefore, that the comparison of the coefficients of variation should be made with appropriate reservations.

The assumption of normality of distribution of error has been made above in establishing the range of error that might be expected in a wide experience. The universal application of this hypothesis has been substantially questioned in recent years by a few workers who have demonstrated its inaccuracy in several illustrative cases. It is advisable then to consider the validity of the hypothesis in the present connection.

Figures 1, 2, and 3 present histograms for the actual distribution of error in the protein, moisture, and ash analyses respectively. Graduation of these errors has been made in each case by the "normal" curve (full line) and the appropriate Pearsonian curve of the non-normal types (broken line). The χ^2, P criteria for goodness of fit of the curves are given in Table VI. In each case it will be seen: (1) That the Type VII curve appears to provide a better graduation of the data than the "normal" curve; (2) that both types of curves are symmetrical, i.e., that errors of the same magnitude but opposite sign appear to occur with equal frequency; (3) that very small and very large errors appear to be of greater frequency than the "normal" distribution would allow for. However, while the Type VII curves provide better graduations of the data than the "normal" curves, they are not themselves entirely satisfactory in all cases. While the fit

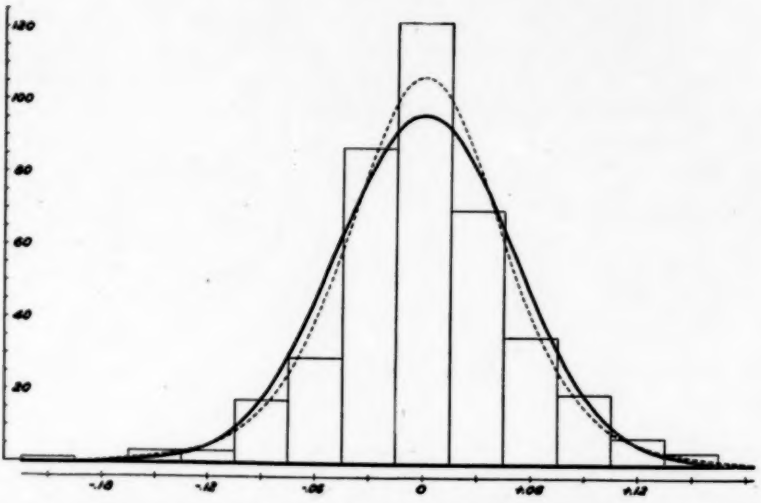

Fig. 1. The distribution of errors within laboratories for protein content of flour. Histogram presents 396 individual errors for 99 collaborators. The full line is the fitted "normal" curve; the broken line the symmetrical Pearson Type VII curve suggested by the moments as the curve of best fit.

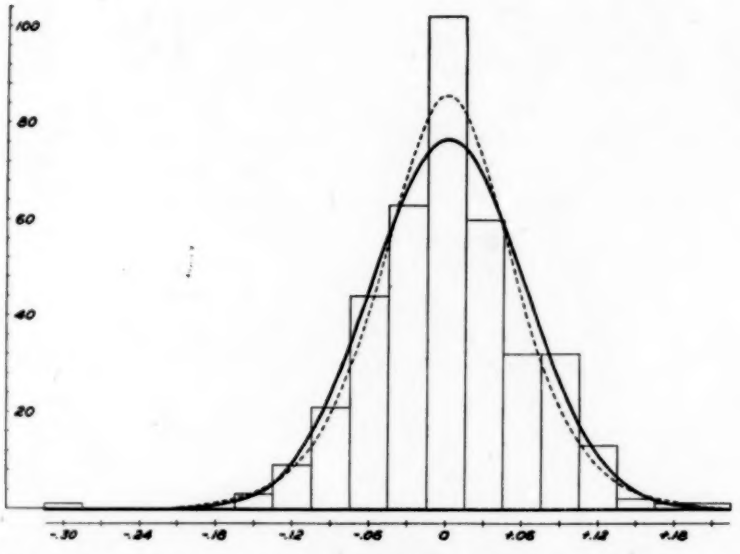

Fig. 2. The distribution of errors within laboratories for moisture content of flour. Histogram presents 384 individual errors for 96 collaborators. The full line is the fitted "normal" curve; the broken line the symmetrical Pearson Type VII curve suggested by the moments as the curve of best fit.

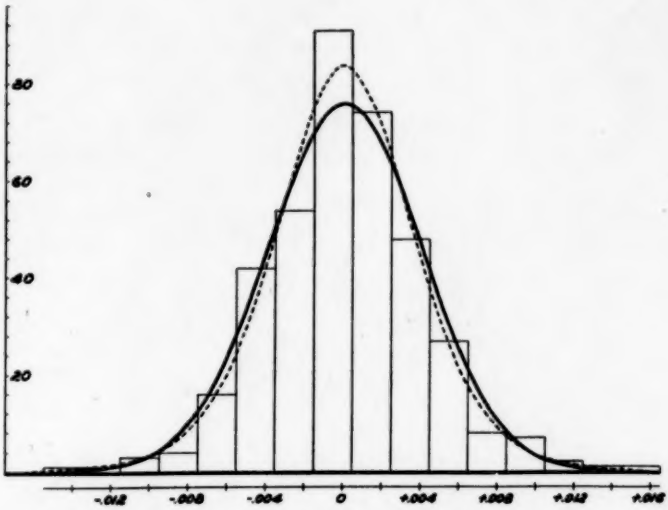

Fig. 3. The distribution of errors within laboratories for ash content of flour. Histogram presents 380 individual errors for 95 collaborators. The full line is the fitted "normal" curve; the broken line the symmetrical Pearson Type VII curve suggested by the moments as the curve of best fit.

TABLE VI

CRITERIA OF GOODNESS OF FIT OF THE DISTRIBUTION CURVES IN FIGURES 1, 2, AND 3

Determination	Normal curve				Pearson non-normal curve				
	d/f	χ^2	*P*	Range[1]	Type	*d/f*	χ^2	*P*	Range[1]
Protein	6	20.5	.002	.22%	VII	8	13.7	.088	.28%
Moisture	8	17.7	.024	.27%	VII	9	17.8	.038	.35%
Ash	8	16.5	.036	.018%	VII	11	7.7	.738	.023%

[1] Central 99% of the area in each case.

is excellent for the ash series, it is sufficiently poor for the moisture series to warrant its rejection, while for protein it does not fit as well as might be desired. There would appear to be some factor of heterogeneity in the data precluding its uniform graduation by an established frequency function.

It might be expected in advance that the precision of analytical determinations would vary from one laboratory to another, or from analyst to analyst, depending on the equipment each employs, the demands made of the analysts, and their experience in the work. In such an event the distribution of standard deviations of errors for collaborators in the present series would not conform to the theoretical curve from samples of 4 only randomly differentiated in that respect. To test this matter, the distribution of standard deviations, actual and theoretical (the latter based on the values σ_∞ in Table IV), have been

drawn and are presented as the histograms and curves of Figure 4. The non-agreement between the histogram and the curve is apparent at once in every case, being markedly greater for ash than for protein, with moisture falling between these two extremes. The discrepancies

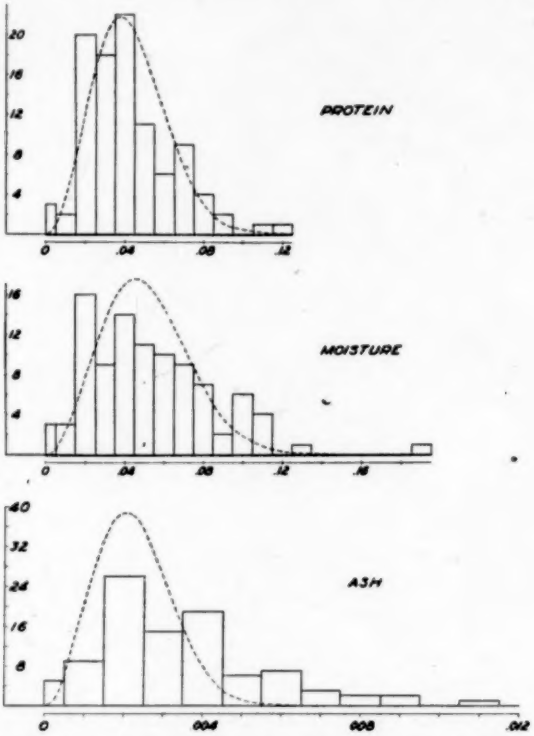

Fig. 4. The distributions of standard deviations for quadruplicate analyses. Actual distributions are given by the histograms. Theoretical expectancy, assuming individual standard deviations to differ from the average by errors of random sampling only, is given by the curve in each case.

(deficiency in the central region and excess at the tails) are exactly of the character that would be expected if real differentiation in the accuracy of collaborators' analytical work existed. This is a matter of important consequences, not only in fixing standards of error for these analytical procedures, but also in determining a generalized form of distribution of error in each case.

Considering first the latter problem, it follows that if the distribution of error for individual collaborators be "normal" but the standard deviations of those errors be differentiated, varying continuously from characteristically small errors to larger errors, then the composite curve of the errors of all collaborators combined would not be "normal" but of the Pearson Type VII. This is precisely the result which appears in the present study. While it cannot be asserted conversely

that therefore the distribution of error within laboratories in general is "normal," it does follow that the distribution of error is less leptokurtic (i.e., peaked in the center and extended at the tails) than is given by the broken line curves of Figures 1 to 3. The writer suggests, then, that at the present stage of the enquiry into the distribution of error in these analytical procedures the hypothesis of normality of distribution of error within laboratories will provide a satisfactory basis of deduction for practical purposes. Moreover, such an hypothesis will have a decided advantage of ease of application far exceeding its alternatives. Any error it introduces must be small and would probably be negligible in its influence.

What standard deviation then, may advisedly be accepted *pro tempore*, as a basis for fixing range of error in protein, moisture, and ash determinations on flour as conducted at present by members of the American Association of Cereal Chemists? Since laboratories differ among themselves in accuracy, should the best, or the worst, or some intermediate value of accuracy be selected as a standard?

In summarizing a series of varying quantities, the human mind has long sought refuge in the mean as the most satisfactory representative or typical measure of the series. There is much to commend that representative measure as the basis of standards in the present problem, despite an idealistic urge to choose a value of lower magnitude. Accordingly the error ranges for protein, moisture, and ash (0.28%, 0.33%, and 0.022%, respectively) as given for the "normal" curve in Table IV are presented here as the limits within which *single* analyses are essentially in accord. They may, then, be regarded as the permissable "tolerance" in each case between results of single analyses.

It is well known that the accuracy of a mean increases in proportion the square root of the number of measures on which it is based. Thus the "tolerance" between averages of duplicate analyses would become 0.7071 times that of single analyses, or 0.20% for protein, 0.23% for moisture, and 0.016% for ash. It follows directly that for these error ranges to be reduced to 0.1% for protein and moisture, and 0.01% for ash, the averages of (2.8^2) 8, (3.3^2) 11 and (2.2^2) 5 determinations, respectively, must be secured. Surely it is clear that the reporting of these analyses to fractions beyond 1/10th, 1/10th and 1/100th of one per cent, respectively, represents entirely unwarranted and unjustifiable refinement for commercial work unless averages of the number of determinations required above are used.

(2) *Systematic Errors of Analysis*

Random errors, so designated because combinations of factors governed by chance alone determine their magnitude in any given instance, are uncontrolled errors and, in part at least, are uncontrollable. Their extent may, by careful attention to details of technique, be reduced in some cases. It must prove entirely impossible to completely eliminate them, however, for, as Gibbs (1929) has expressed the principle, "No measurement of any real thing can ever be correct, for the simple reason that no instrument is capable of infinitely small displacements and no human eye can detect infinitesimal separations. Errors are, therefore, inevitable."

Of an entirely different character are the systematic errors of analysis, which repeat themselves to the same degree in replicating the same work so long as the technique is not changed. Such errors are the product of fixed conditions, and their magnitudes may be determined and corrected for by appropriate measures. Essential to their elimination, however, is a frank recognition of the plausibility of their occurrence in one's own work equally with that of others. In the absence of *absolute* knowledge of the percentage occurrence of a constituent being determined, it is impossible to determine the magnitude of one's systematic error except through cooperation with other laboratories conducting analyses of the same material. When the determinations of a large number of analysts are available, the average of all analyses which appear to have equal validity should be accepted as the best estimate of the "true" value of the unknown constituent. Consistent deviation from such "true" averages by individuals reporting may be accepted as a measure of their respective systematic errors. They should be corrected for accordingly, preferably by critical examination of the technique of analysis to remove them at their source.

In view of the precision which traditionally belongs to quantitative chemistry, it is surprising indeed to see the extent to which such systematic errors may render untrustworthy the reports upon such routinely determined consitutents as protein, moisture, and ash in flour. Illustrative examples are given in the results of the present investigation, wherein an endeavor has been made to ascertain the true value of these constituents in a carefully sampled flour by consulting approximately 100 laboratories for their verdicts.

Since averages of N replicates must be expected to vary over a range of error $N^{-1/2}$ times the range of error of the individual analyses —and this will be true independently of who performs the analyses— means of four determinations of protein, moisture, and ash reported by different analysts using the same sample of flour may be expected to

vary over half the range found valid for the individual determinations. Any greater variation than this between observed averages of 4 determinations in a collaborative study must be due to the presence of systematic errors or bias in chemical analyses differentiating the reports of analysts one from the other.

Working in terms of the standard deviations of collaborators, the *maximum* expected ranges for the averages of their reports would become .31% protein, .49% moisture, and .028% ash. These values are derived from the highest individual standard deviations of collaborators—numbers 15, 113, and 90—and, therefore, would be the valid ranges if all sets of analyses showed equally great discrepancies of replication. Against this the actual distribution of averages, represented by the histograms in Figures 5 and 6, show ranges of 3.6%

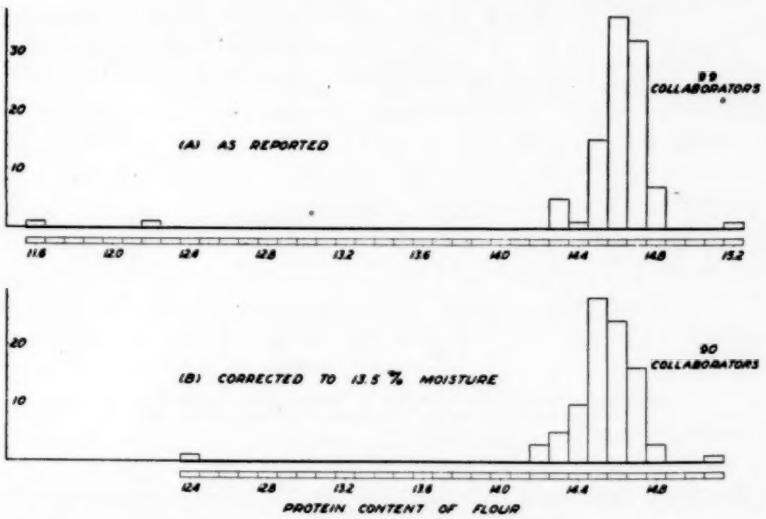

Fig. 5. Actual distributions of the averages of quadruplicate analyses for protein in flour sample 173, (A) as reported by collaborators, and (B) corrected to a uniform moisture basis. The theoretical range for such averages in the absence of systematic errors equals 0.14 units (see text).

protein, 2.7% moisture, and 0.8% ash. Thus the *range of systematic error* in the present series of analyses is 3.3% for protein, 2.2% for moisture, and 0.77% for ash. These are surely astounding results. It will be protested immediately that they are largely due to a few highly discrepant workers and do not reflect the general situation. Let us then reject the "worst" or extreme cases of 11.6%, 12.2%, and 15.2% for protein, 12.4% and 15.1% for moisture, and .44% and .455% for ash. The resulting selected groups, of quite orderly and compact distribution in each case, show actual ranges of averages of 0.5% for protein, 0.8% for moisture and 0.055% for ash, values still

indicating that the *minimum* ranges of systematic error between laboratories are of the order of 0.2% for protein, 0.3% for moisture and 0.03% for ash.

The above comparisons provide the absolute minimum ranges of systematic error derivable from the data. They are open to the criticism of showing selection of the records to give the best appearance (i.e., favoring absence rather than presence of systematic error).

If the average random error of laboratories (corrected for size of sample) be used instead of the maximum in each case for fixing toler-

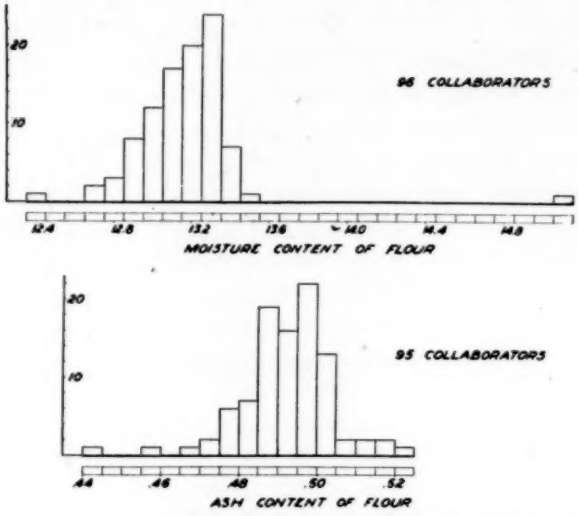

Fig. 6. Actual distributions of the averages of quadruplicate analyses for moisture and ash n flour sample 173. Theoretical ranges in the absence of systematic errors would be 0.17 and 0.011 units respectively (see text).

able discrepancies—and this seems to be the more appropriate procedure, these ranges of *intolerable* systematic error between laboratories become approximately 0.36% for protein, 0.63% for moisture, and 0.044% for ash, each one considerably greater than the average *random* error within laboratories. Remembering that these results are based upon the rejection of the "worst" cases of systematic error, it becomes clear that *the major cause of discrepancy between laboratories in general, reporting on protein, moisture, and ash in flour, is not the random error or inaccuracy of the method of analysis, but the avoidable systematic error.*

Conclusions

In this study of from 95 to 100 independent reports from collaborators performing analyses in quadruplicate for the percentage of protein, moisture, and ash in a carefully prepared sample of a "typical"

bakers' patent flour, it has been shown that two important and distinct classes of error characterize the results. These are the uncontrolled and perhaps unavoidable *random errors* of analysis, and the controllable *systematic errors* differentiating laboratories. The latter appeared in this investigation to be even more important than the former, being markedly so in certain extreme cases.

Variation in the precision of analyses as reflected in the standard deviations of the results of collaborators is shown to be of such a character as to establish significant differentiation of the analysts in regard to precision of their work. This differentiation is but small for protein analyses, greater for moisture determinations and quite pronounced for ash determinations.

"Normality" of distribution of the errors characterizing the work of individual analysts appears to be approached with sufficient closeness to justify use of the "normal" curve in establishing error ranges from known standard deviations.

The average standard deviations of quadruplicate analyses for all collaborators being, respectively, 0.0427%, 0.0513%, and 0.0034% for protein, moisture, and ash in the flour sample employed, it follows that the ranges of the central 99% of infinite series of analyses on this flour sample would approximate to 0.28% for protein, 0.33% for moisture, and 0.022% for ash. To reduce the error ranges to 0.1%, 0.1%, and 0.01%, respectively, it would appear from the above results that the averages of 8 determinations would be necessary for protein, 11 for moisture, and 5 for ash. Clearly then, for commercial purposes at least, it is foolish to quote these constituents to smaller fractions of one per cent unless averages as above are secured.

Systematic errors of analysis characterize the results of laboratories in the form of a bias. Such errors being clearly demonstrated to be even greater in magnitude than the random errors, the application of effort toward their removal must commend itself. This can only be accomplished through collaborative effort and frank recognition of the equal claim to validity of the work of analysts so collaborating, clearly exceptional cases as occur in this study being permissably excluded.

Stress must be given to the fact that the above results flow from a study of but *one* sample of flour. Experience indicates that the extent of random error is a function of the characteristics of the flour sample as well as of the method of analysis. *A priori* reasoning would suggest that, in general, there would be a simple and direct (though almost certainly less than proportional) variation in the extent of the random error of analysis with variation in the magnitude of the constituent being determined. A positive correlation may be expected between the magnitude of the random errors of analysis and the true value of the

unknown constituent. Hence the standards of random error suggested by this study may be too lenient for some flours, and not lenient enough for others. The solution of this problem must remain for further study.

It is recommended by the Committee on Methods of Analysis of the American Association of Cereal Chemists that the ranges of random error given above be recognized by the Association *pro tempore* as a permissable "tolerance" between single analyses for those constituents of flour.

Acknowledgments

The writer is indebted in large measure to the analysts who cooperated in this study; also to C. E. Mangels who, as Chairman of the Committee on Methods of Analysis, officially sponsored the investigation. To them, and to the writer's assistants who labored at the task of statistical computation, he offers sincere thanks.

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A WHOLE WHEAT MEAL VISCOSITY TEST FOR DETECTING CERTAIN TYPES OF POOR MILLING WHEATS

E. G. BAYFIELD [1]

(Read at the Convention, May, 1932)

New varieties of wheat are constantly coming on the market and becoming available in commercial quantities to the miller. Occasionally, these new varieties become widespread on account of their excellent agronomic characteristics but later they prove unsuitable to the miller on account of their lack of ability to produce a large yield of flour of a low ash content. Such wheats, once established, cause considerable loss to the milling industry and their removal from the trade requires many years of effort and propaganda. Wheats of this type should be eliminated while the variety is in the hands of the wheat breeder.

At the present time there are no very satisfactory methods for evaluating the flour-yielding ability of a wheat when only a relatively small amount of grain is available. With very small quantities of grain the experimental milling test is of uncertain value; and besides it is very time consuming. Under ideal conditions the experimental milling test requires a considerable amount of grain from the plant breeder's viewpoint.

The ash content of flour is a commonly used criterion of its quality. In commercial practice millers try to maintain a uniform ash content in their products. Sherwood (1926) found a high positive correlation between the ash content of the wheat and the ash content of the straight grade flour milled from the wheat. A high-ash wheat may produce a low-ash flour, but only by using a shorter extraction. However, a number of factors, such as tempering, source of supply, grade, variety of wheat, or the type of mill, prevent the percentage of ash in wheat from being used as a measure of flour-yielding ability.

Morgan (1924) used wheat meal and the MacMichael viscosimeter as a basis for purchasing hard winter wheat. Durham (1925) used a similar type of determination and found that large differences existed between different market classes of wheat. Durham (personal communication, 1931) stated, that for a time he used his viscosity test as an aid in blending his mill mix. Herman (1926, 1927) used Durham's

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(1925) viscosity test as one of his analytical procedures in studying the baking characteristics of different varieties grown in a uniform environment.

Any test to be particularly useful as an aid to mill control or to the plant breeder must be reasonably easy to carry out and also fairly rapid, as large numbers of samples would, of necessity, have to be handled. It appeared as though a test somewhat similar to that described by Durham (1925) might prove of considerable value for determining the milling quality of the soft winter wheats of Ohio. Preliminary studies indicated, however, that such a test would have to be considerably modified before proving satisfactory for these Ohio wheats.

Experimental

Grinding the Sample

The size of the particles in a suspension is of prime importance in any viscosity determination. The method of grinding a sample, therefore, becomes important. If the particle size is too large, settling occurs in the viscosimeter during the test. Overgrinding in some types of machines produces excessive heat in the sample, which is liable to produce possible changes in the colloidal condition of the wheat meal. The influence of grinding equipment was, therefore, studied. Two grinders, of the burr type, and a small hammermill were available for studying the effect of grinding.

An Enterprise No. 059 grinder was tried first. This machine proved unsuitable because the sample balled-up when fine grinding was attempted. With both burr grinders heating occurred and the bran fragments were too large in size. The second machine, a Grusonwerk also of the burr type, was of German manufacture. This grinder, when set close enough, proved suitable insofar as size of particle was concerned, but at this setting excessive heating took place after the grinder had run but a short time. A small sized Jay-Bee hammermill, using a 1/32-inch sized sieve, proved capable of grinding a suitable sample and gave duplicate grindings which checked reasonably well from a viscosity standpoint. However, it was necessary to feed the wheat into the mill at a uniformly low rate of speed. In addition, this machine proved difficult to thoroughly clean out and a portion of the sample was lost during the cleaning process. Nevertheless, for the purposes of this study all grindings were made on this mill.

As might be expected, varieties ground differently, due to the fact that some varieties are harder and more vitreous than others. Three varieties grown under identical environment in 1931 were ground in duplicate during part of a day's grinding. Red Rock, Trumbull, and

American Banner were selected. Red Rock (No. 984) is a standard "strong" soft red winter variety; Trumbull (No. 981) is medium in strength; whereas American Banner (No. 985) is a weak soft white winter. Due to growing conditions, less spread in strength existed between these varieties in 1931 than is ordinarily the case. However, they were used during the preliminary tests before a standardized technique was adopted. After grinding, each sample was run through a nest of sieves consisting of an 18 wire, a 64-grits gauze, and a 12 XX silk. The 18 wire removed any large sized particles accidentally present. These were discarded, since they were negligible in amount. The overs and thrus from the other two sieves give an approximate idea as to how the varieties ground down.

From Table I it may be seen that the softer variety American Banner (No. 985), produced the most flour (through 12XX), and that

TABLE I

RESULTS FROM DUPLICATE GRINDINGS OF THREE VARIETIES OF WHEAT

Sample number	Percentage of meal obtained [1]			Viscosity in degrees MacMichael		
	Over 64 GG	Over 12 XX	Thru 12 XX	No acid	+ acid	+ H_2O_2
984-F	26.2	24.7	48.6	102	131	176
984-G	23.7	24.1	51.8	106	133	174
981-F	22.1	21.9	55.5	95	138	201
981-G	17.4	22.7	59.5	98	139	199
985-F	18.2	23.3	57.9	59	90	143
985-G	17.4	21.1	61.1	60	90	142

[1] Total percentage less than 100, due to loss during sifting.

the variety Red Rock (No. 984) produced the least flour. The variety Trumbull occupied an intermediate position. No reason is apparent for the more thorough pulverization which occurred in the G grindings as compared with the F grindings. The procedure followed in the above viscosity determinations will be given later.

The Viscometric Determination

Durham (1925) used 30 gms. of meal in 100 cc. of distilled water, whereas Morgan (1924) used 20 gms. with 65 cc. of water. Ohio wheat ordinarily contains less protein than Kansas hard winters, and preliminary tests indicated that 30 gms. of meal did not produce a suspension of a suitable mobility when suspended in 100 cc. of water. After many tests 35 gms. of meal were finally decided upon for wheat containing 9 to 11% of protein. As no significant difference existed in the moisture contents of the samples under study (all containing about 10% of moisture) no correction was made for moisture. Correction for

moisture should prove desirable under conditions where considerable variation exists in the moisture content of the different samples under test.

Durham (1925) used 2 cc. of concentrated lactic acid in his determinations. A considerable amount of work with concentrated lactic acid (85%) indicated that between 1.4 and 2.0 cc. of this concentrated acid produced a reasonably stable suspension. Gortner and Sharp (1923) found that various acids produce their maximum imbibition effects at a hydrogen ion concentration of, roughly, pH 3.0.

It seemed desirable, therefore, to check electrometrically, the effect of varying increments of concentrated lactic acid. A quinhydrone electrode was employed. The suspension consisted of 35 gms. of Trumbull wheat meal suspended for 1 hour at 86° F. in 100 cc. of liquid consisting of sufficient distilled water and acid to make this volume. The suspension without acid gave a pH reading of 6.13. The use of 1.4 cc. of acid produced a pH of 3.16, whereas 2.0 cc. gave a suspension of pH 2.94. Thus, increasing the amount of acid by 0.6 cc. caused an increase in acidity equal to 0.22 pH. During this change the "+ acid" viscosity reading increased 3°, or from 128° to 131° MacMichael. The addition of 2 drops of 3% hydrogen peroxide ("+ peroxide" reading) to these same two samples produced a further gain of 1° MacMichael for the more acid suspension. This change in viscosity (from 176° to 177°) is too small to be significant.

The effect of using still more acid suspensions was interesting. The use of 2.6 cc. of acid gave a pH of 2.78, a "+ acid" viscosity of 128°, and a "+ peroxide" viscosity of 167° MacMichael. Three cubic centimeters of acid gave a pH of 2.71, a "+ acid" viscosity of 138°, and a "+ peroxide" reading of 163° MacMichael. The most acid suspension used contained 3.4 cc. of acid, possessed a pH of 2.60, had a "+ acid" reading of 137°, and a "+ peroxide" reading of 142°. Thus, it is seen that the use of much over 2.0 cc. of acid tended to produce a rather unstable suspension with acid alone, and the effect of the hydrogen peroxide was rapidly suppressed as the acidity increased. In all of the above suspensions the meal was first mixed into a rather thick paste, using distilled water. To this paste the remainder of the 100 cc. of liquid which contained the required amount of acid was added. It was found that the meal gave as much as 25% lower viscosity readings if it was suspended directly in acidulated distilled water instead of being moistened and then acidulated.

In the baking test it has been found desirable to use machine mixing to reduce variability in results. Similarly, making the flour-in-water suspensions by machine proved superior to mixing by hand in a beaker or by mixing with a mortar and pestle. An electric stirrer similar to

those used commonly at soda fountains was employed. The stirring rod was equipped with a brass hexagonal plate approximately 3/4 inches in diameter and about 3/8 inches in thickness. The motor and stirrer normally operated at about 2300 r. p. m. This speed, however, caused excessive foaming. By placing a 60-watt tungsten lamp in series with the motor, the speed was very considerably reduced and the production of foam largely eliminated. Foaming was also reduced by having the stirrer immersed as deeply as possible in the suspension. The standardized procedure finally adopted as a result of the several preliminary experiments follows. When strictly adhered to, duplicatable results obtain.

Method used in Study

From an automatic burette, 50 cc. of distilled water were measured into a 250 cubic centimeter beaker; 35 gms. of meal, previously weighed out, were then poured into the water and stirred by machine for 2 minutes. A glass stirring rod was used simultaneously during the stirring process and the beaker was slowly rotated by the left hand. Upon completion of the 2-minute period, the beaker was removed from the machine and stirred further by hand to reduce any lumps which might have escaped the previous stirring action. Following this, in the case of the "no acid" determinations, a second 50 cc. of distilled water were run into the suspension, stirred by hand and placed in a water bath held at 86 ± 1° F. for 1 hour. The "+ acid" determinations received 50 cc. of acidulated distilled water which contained 1.7 cc. concentrated lactic acid (85%) per 50 cc. of solution. A second automatic burette was used for measuring out the acidulated solution. The suspension was then treated in the same manner as the "no acid" samples. While in the water bath, samples were stirred regularly about every 10 minutes. In running a series of determinations the time required for operating the tests on the MacMichael viscosimeter regulates the interval which must occur between mixing different samples. Ordinarily, 5-minute intervals were required for "no acid" determinations and 9-minute intervals for the "+ acid" and "+ peroxide" tests.

The viscosimeter test was performed on the MacMichael viscosimeter, using the large, flat, disk bob, a No. 30 wire, and a bowl speed of 20 r. p. m. The room temperature was regulated as closely as possible to 86° F. However, variations in air temperature of as much as ± 5° were occasionally noted. The suspension was poured into the viscosimeter bowl and stirred thoroughly with the bob, after which the the bob was suspended and the motor started. Some practice was required before knowing when the equilibrium point was reached in

the "no acid" determinations, as settling out of the larger particles caused drifting to occur. A short pause ordinarily marked the best point for taking the reading. The bob was then removed and the sample stirred with it for the second time and the reading taken again. If it checked with the first reading within 2° MacMichael, the test was completed; otherwise, a third stirring and reading were made. The bowl and bob were then washed and dried.

The "+ acid" suspensions were handled exactly as the "no acid" samples, with the exception that, instead of discarding the suspension upon obtaining a satisfactory reading, two drops of 3% hydrogen peroxide were added. The suspension was then thoroughly and continuously stirred during a definite time period. The time was measured by allowing the viscosimeter bowl to rotate 6 revolutions during the stirring process. The viscosity readings were then obtained as before, except that they were repeatedly taken until a maximum measurement was obtained, each reading being preceded by a stirring. With good milling samples this maximum, ordinarily, was the first reading. The peroxide acted less rapidly on those samples considered as poor milling wheats, i.e., those which gave high-ash flours. The results from these samples were the most difficult to duplicate and required several readings before obtaining a maximum reading. With peroxide, a difference of 4° to 5° MacMichael between duplicates was considered a satisfactory check. The above procedure was employed uniformly in testing all the samples mentioned in the following discussion.

Source of Material

All wheat samples used were sub-samples of the Ohio collection made for the Tri-State Soft Wheat Improvement Association (Bayfield, 1931–a). The 1930 crop samples were grown at Wooster, Ohio, and those for 1931 were produced at Wooster and in other parts of the State. Analytical data for percentages of ash, crude protein, and loaf volume were obtained from records of the Association (published in part by the Tri-State Soft Wheat Improvement Association, 1931, 1932). All ash and crude protein results are given on a 15% moisture basis.

Results and Discussion

Table II gives the viscosity results for certain standard soft winter wheats when grown at Wooster. Three of these, American Banner, Dawson's Golden Chaff, and O. A. C. No. 104, are white wheats, weak in strength, but producing a good yield of flour. They are not typical bread wheats. The red winter wheats, Purkof, Michikof, and Berkeley Rock, have proven in out tests to mill with difficulty and to produce a lower yield of flour than the standard variety, Trumbull. In addition,

TABLE II

VARIETAL BEHAVIOR IN THE WHEAT MEAL VISCOSITY TEST (ALL VARIETIES GROWN AT WOOSTER, OHIO)

Variety and year		Viscosity in degrees MacMichael			Increase in viscosity with	
		No acid	+ acid	+ H_2O_2	Acid only	Acid and H_2O_2
Trumbull	1930	72	114	185	42	113
	1931	95	129	185	34	90
	Average	83	121	185	38	102
Nabob	1930	59	91	129	32	70
	1931	63	81	125	18	62
	Average	61	86	127	25	66
Fulhio	1930	69	108	161	39	92
	1931	90	138	202	48	112
	Average	78	119	181	41	103
Red Rock	1930	86	142	198	56	112
	1931	94	124	166	30	72
	Average	90	133	182	43	92
Michigan	1930	82	130	195	48	113
Amber	1931	81	128	184	47	103
	Average	81	129	189	47	108
Fultz	1930	77	121	165	44	88
	1931	87	127	180	40	93
	Average	82	124	172	42	90
Gladden	1930	79	106	150	27	71
	1931	94	114	150	20	56
	Average	86	110	150	24	64
Kharkov	1930	82	116	163	34	81
	1931	90	128	180	38	90
	Average	86	122	171	36	85
Berkeley	1930	106	176	231	70	125
Rock	1931	112	173	232	61	120
	Average	109	174	231	65	122
Purkof	1930	110	184	274	74	154
	1931	116	178	243	62	127
	Average	113	181	258	68	145
Michikof	1930	124	197	298	73	174
	1931	113	176	251	63	138
	Average	118	186	274	68	156
American	1930	56	80	123	24	67
Banner	1931	62	89	142	27	80
	Average	59	84	132	25	73
Dawson's	1930	60	89	144	29	84
Golden	1931	70	96	158	26	88
Chaff	Average	65	92	151	27	86
O. A. C.	1930	61	104	156	43	95
No. 104	1931	66	98	146	32	80
	Average	63	101	151	38	88

these three varieties produce a high-ash flour even when grown under the same environment as other varieties. Their milling behavior ranks them as do the viscosity results, a high increase from adding acid or acid and peroxide signifying high-ash flours and low flour yields. Rather limited milling data seem to substantiate the relative ranking of the remaining varieties as determined by the wheat-meal test.

Having obtained the above results, it seemed desirable to study the relationships existing between the wheat-meal viscosity test and other

standard tests, such as protein content and loaf volume. To do this, a series of 50 samples was ground and the test performed on them. These samples consisted of a series of 10 varieties, each series having been grown at five locations over the State in 1931. Table III gives the viscosity results and certain other data regarding these samples. The various headings are self explanatory, with the exception of "patent flour ash." The figures in this column refer to the ash content of the flour designated as "patent" from experimental milling. In the case of these samples, the weight of the patent flour equalled 60% of the weight of the tempered wheat (15.5% moisture content) milled. The loaves were baked from flour with an ash content of approximately 0.45%. Thus, for example, in the case of sample No. 911 sufficient of the low-grade flour was mixed with the "patent" flour to bring the ash content to the required level and this blended flour was then used in baking.

The averages for the samples in Table III rank the varieties in an order somewhat different from that of Table II but the agreement is reasonably good. Berkeley Rock gives the largest increase in viscosity with acid or acid plus peroxide and it is the poorest milling wheat of the 10 varieties concerned. Nabob and Gladden give the least increases when grown at one location or when considered as averages from several locations.

It will be observed from the data in Table III that the protein content tends to increase with the magnitude of the viscosity readings. Similarly, the viscosity tends to increase with increasing ash in the patent flour. In commercial practices when the ash content runs too high, it is commonly reduced by decreasing the extraction. Accordingly, with a variety like Berkeley Rock, if the extraction had been lowered, then a lower ash in the patent flour should have resulted. Ash in the patent flour obtained with a uniform extraction should, therefore, be a rough measure at least of the patent flour-yielding ability of the variety concerned. Since the wheat meal viscosity with acid or acid and peroxide increases with the ash in the flour, a low viscosity rating indicates a high flour-yielding ability. This is in agreement with the results obtained in experimental milling of the samples.

In order to study the relationships existing between the viscosity test and the regular tests employed on the samples, the various total correlation coefficients in Table IV were computed. Most of the coefficients are low in magnitude but the majority are significant when considered in relation to their probable errors (a coefficient being considered significant when it is as great as, or greater than, three times its probable error). Table IV indicates that both the crude protein content of the wheat and the ash in the patent flour bear a direct rela-

TABLE III

RESULTS FROM TEN VARIETIES GROWN AT EACH OF FIVE LOCATIONS

Serial number	Ash in patent flour	Crude protein in wheat	Loaf volume	Test weight	Viscosity in degrees MacMichael			
					No acid	+ acid	+ H_2O_2	Total increase [1]
	P.ct.	P.ct.	Cc.	Lbs.				
				Variety—Trumbull				
911	0.382	9.3	470	59.4	65	86	120	55
941	.407	10.8	465	59.7	92	134	191	99
961	.428	10.5	440	59.7	90	124	178	88
981	.483	10.1	430	57.4	95	129	185	90
991	.484	10.1	442	57.4	89	134	186	97
Average	.438	10.2	449	58.7	86	121	172	86
				Variety—Nabob				
912	.430	8.5	385	59.2	56	78	100	44
942	.396	9.8	450	59.6	69	89	130	61
962	.459	9.5	428	60.1	64	87	134	70
982	.459	9.3	450	57.3	63	81	125	62
992	.612	9.4	415	60.1	76	105	138	62
Average	.471	9.3	426	59.3	66	88	125	60
				Variety—Fulhio				
913	.408	9.5	430	59.8	72	112	156	84
943	.436	10.5	495	60.0	84	130	202	118
963	.452	9.8	440	59.6	92	132	199	107
983	.462	10.2	470	57.2	90	138	202	112
993	.613	10.4	405	57.4	104	162	222	118
Average	.474	10.1	448	58.8	88	135	196	107
				Variety—Red Rock				
914	.493	9.3	420	59.5	88	132	184	96
944	.456	10.1	490	59.5	93	124	147	54
964	.499	9.5	430	60.5	96	130	175	79
984	.486	9.9	462	57.6	94	124	166	72
994	.461	9.6	420	58.5	102	145	188	86
Average	.479	9.7	444	59.1	95	131	172	77
				Variety—American Banner				
915	.435	8.3	390	56.7	57	86	138	81
945	.446	9.3	470	57.3	60	90	124	64
965	.443	9.4	410	58.3	61	95	140	79
985	.434	8.9	432	55.3	62	89	142	80
995	.428	9.0	420	55.7	78	112	161	83
Average	.437	9.0	424	56.7	64	94	141	77
				Variety—Berkeley Rock				
916	.629	8.5	405	59.5	88	138	208	120
946	.579	10.4	440	60.3	116	174	247	131
966	.639	9.9	350	61.0	102	156	233	131
986	.553	10.1	427	58.2	112	173	232	120
996	.535	9.6	435	59.3	110	153	203	93
Average	.587	9.7	411	59.7	106	159	225	119

TABLE III—*Continued*

Serial number	Ash in patent flour	Crude protein in wheat	Loaf volume	Test weight	Viscosity in degrees MacMichael			
					No acid	+ acid	+ H_2O_2	Total increase [1]
	P.ct.	P.ct.	Cc.	Lbs.				
				Variety—Michigan Amber				
917	.476	8.6	380	57.5	75	108	172	97
947	.473	11.0	472	59.9	88	120	161	73
967	.474	9.9	380	59.3	89	128	189	100
987	.503	10.6	457	56.4	81	128	184	103
997	.496	10.2	440	58.5	90	131	184	94
Average	.484	10.1	426	58.3	85	123	178	93
				Variety—Kharkov				
918	.544	8.8	430	59.3	67	99	148	81
948	.536	10.3	450	60.6	93	122	175	82
968	.570	9.9	410	59.8	88	113	155	67
988	.623	10.3	450	56.6	90	128	180	90
998	.494	9.7	425	58.6	89	115	138	49
Average	.553	9.8	433	59.0	85	115	159	74
				Variety—Fultz				
919	.517	9.3	440	59.4	78	116	173	95
949	.437	9.7	420	60.0	105	138	201	96
969	.457	9.8	410	59.2	93	128	191	98
989	.468	9.7	440	56.4	87	127	180	93
999	.557	10.2	427	57.5	114	146	205	91
Average	.487	9.7	427	58.5	95	131	190	95
				Variety—Gladden				
920	.461	8.4	422	59.1	70	92	133	63
950	.404	9.1	447	60.5	87	109	142	55
970	.395	9.2	425	60.3	84	110	154	70
990	.451	9.5	425	57.6	94	114	150	56
1000	.514	9.2	387	58.1	87	104	127	40
Average	.445	9.1	421	59.1	84	106	141	57

[1] "Total increase" is the total increase in viscosity due to adding acid and peroxide.

TABLE IV

Correlation Coefficients

Total correlation between	Viscosity readings in degrees MacMichael			
	No acid	+ acid	+ H_2O_2	Total increase [1]
Crude protein (wheat)	+.5821±.0631	+.5849±.0627	+.5260±.0690	+.3759±.0819
Ash (patent flour)	+.4521±.0759	+.5189±.0697	+.4951±.0720	+.4191±.0786
Loaf volume	+.0077±.0954	−.0080±.0954	−.0575±.0951	−.0899±.0946
"No acid" reading		+.9219±.0143	+.8104±.0327	+.5087±.0707
"+ acid" reading			+.9454±.0101	
	Ash (flour)	Loaf volume		
Test weight	+.0705±.0949			+.0069±.0954
Crude protein (wheat)	+.1727±.0925	+.4689±.0744		

[1] "Total increase" refers to the increase in viscosity due to adding acid and peroxide.

tionship with all three viscosity determinations carried out on the wheat meal. Of the three determinations, the "+ acid" readings give the highest positive relationships.

Loaf volume is shown as being positively correlated with protein content in the wheat but no significant relationship exists between the wheat-meal test and loaf volume. With the 1930 crop, Bayfield (1931b) obtained a positive correlation ($+.6424 \pm .0265$) between protein in wheat and loaf volume (224 samples), indicating that protein content in soft winter wheat gives a measure of its baking strength. The wheat-meal test, accordingly, gives no information regarding the quality of wheat protein, the strength of which may be measured by the baking test.

Test weight per bushel is a commonly accepted criterion of the milling quality of wheat. However, no significant correlations were obtained on the 1930 crop (Bayfield, 1931b) or in the present study. Between "total increase" in viscosity and test weight, the coefficient equalled $+.0069 \pm .0954$, which indicates a lack of relationship between test weight and the wheat-meal viscosity measurements.

It will be observed that the different viscosity measurements are highly correlated with one another. In other words, a high reading may be expected with the addition of acid and peroxide or with acid alone if a high reading is obtained from the "no acid" determination. In the test in its present stage of development, it would appear as though the "no acid" determinations could be dispensed with. Apparently, the only advantages to be derived from adding peroxide is in an additional spread between the relative magnitudes of readings obtained from the poor- and good-milling wheats. Such a spread is an advantage but it is compensated for, to some extent, by the greater difficulty experienced in obtaining accurate duplicate results.

Summary

A viscosimetric method using suitably ground whole wheat meal has been outlined so that results may be duplicated at will. The procedure is relatively simple and requires a comparatively short time for testing a sample.

Wheat varieties of poor milling quality were found to give much larger viscosity readings than varieties which milled readily and gave low-ash flours.

Statistical studies indicated that this test is positively correlated with the protein content of the whole wheat and also with the ash content of experimentally milled flours obtained from this wheat. No significant relationship existed between test weight per bushel or loaf volume and the viscosity determinations.

The test has been designed primarily for eliminating poor milling varieties while in the hands of the wheat breeder. It may also prove of service in blending soft winter wheats in commercial mill mixes.

Acknowledgements

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REGIONAL AND SEASONAL VARIATION IN PIGMENTATION OF DURUM WHEATS

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(Read at the Convention, May, 1932)

Durum wheats are produced in North and South Dakota, Minnesota, and Montana, and also to a limited extent in the Canadian provinces. North Dakota, however, is the principal durum wheat producing state. In North Dakota, durum wheats are produced principally in the eastern third of the state, but more particularly in the counties lying immediately west of the Red River Valley. Figure 1 shows the distribution of durum wheat acreage in North Dakota.

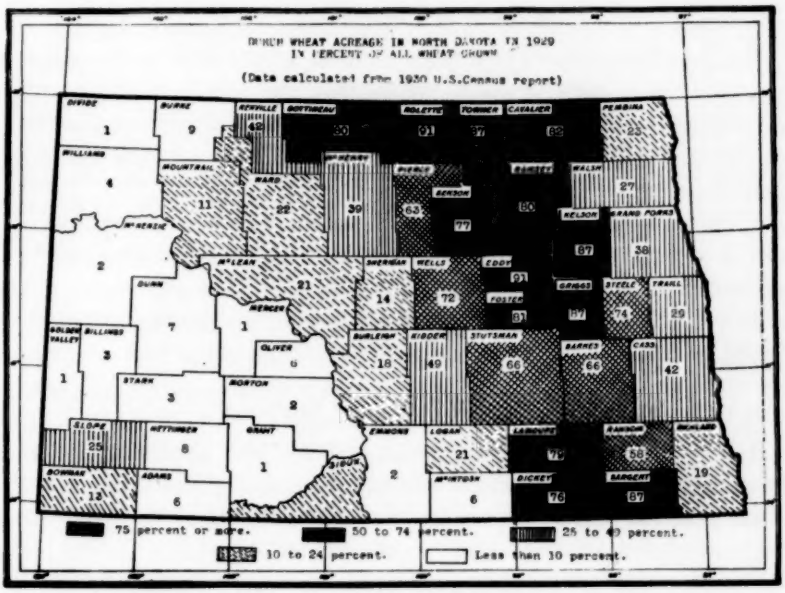
DURUM WHEAT ACREAGE IN NORTH DAKOTA IN 1929
IN PERCENT OF ALL WHEAT GROWN
(Data calculated from 1930 U.S.Census report)
75 percent or more.
50 to 74 percent.
25 to 49 percent.
10 to 24 percent.
Less than 10 percent.

Fig. 1. Distribution of durum wheat acreage in North Dakota in 1929.
(Courtesy of T. E. Stoa, from Circular 46, North Dakota Agricultural Experiment Station.)

The principal outlets for durum wheat produced in this country are semolina production and the export market. Semolina is used in the preparation of macaroni products and the quality requirements, particularly as to color, are quite different from those of bread wheats. For a bread or cake flour, a high degree of whiteness is desirable, but for

semolina or macaroni, a natural rich creamy or yellow color is preferable. The color of durum wheat products is, therefore, an important factor of quality, and this depends largely upon the degree of pigmentation in the original wheat.

Varietal Variation in Color

It has been pointed out previously that different varieties of durum wheat will show considerable variation in color or degree of pigmentation. MacLaren (1923) and Mangels (1923) called attention to varietal variation in color of durum wheats in 1923. Later Coleman and Christie (1926) recorded extensive varietal data on gasoline color values of durum wheats.

While varietal variation is an important factor, it has been observed that the same variety will also show marked variation in color, due to conditions under which the wheat is produced. This paper will consider the possible effects of different factors upon the variation in degree of pigmentation of the same variety.

Method of Comparing Color

The gasoline color value was used as a measure of the degree of pigmentation in this study. While this method is a comparatively crude measure of color, it is well adapted to routine examination of samples and gives fairly accurate data on the relative amount of pigment present.

A 75% patent flour prepared on a small Allis-Chalmers mill was used in all cases for the gasoline color value determinations. The gasoline extracts were prepared by adding 100 cc. of aviation grade gasoline to 20 gms. of patent flour. The extracts were compared with 0.005% potassium chromate solution in a Dubosque type colorimeter.

Effect of Cropping Systems

It is well known that cropping systems and preceding crops will profoundly affect the protein content of wheats (Mangels, 1925). A comparison was made, therefore, of durum wheats of the same variety produced under different systems of crop rotation.

For this study, samples of Mindum durum were secured from the rotation plots at the Langdon Substation.[1] Samples were secured from both the 1930 and 1931 crops and the data taken therefrom are recorded in Table I.

It will be noted that the variation in gasoline color value due to cropping systems is hardly significant. The maximum variation between gasoline color values was 0.32 in 1930 and only 0.13 in 1931.

[1] Through courtesy of Victor Sturlaugson, superintendent of the Langdon Substation.

TABLE I

EFFECT OF CROP ROTATION ON GASOLINE COLOR VALUE OF DURUM WHEATS

Mindum Durum Grown at Langdon

Plate number		Rotation	Gasoline color	
			1930	1931
1	A	Wheat, sweet clover (hay), corn	1.84	1.67
2	B	Wheat, sweet clover (seed), corn	2.00	1.63
3	C	Wheat, sweet clover (plowed under), corn	1.99	1.60
4	D	Corn, sweet clover (hay), wheat	2.00	1.61
5	E	Wheat, wheat, sweet clover (green manure)	2.07	1.65
6	E_x	Wheat, wheat, sweet clover (green manure)	1.85	1.69
7	F	Wheat, wheat, corn	2.00	1.65
8	F_x	Wheat, wheat, corn	2.14	1.71
9	G	Corn, wheat, sweet clover (hay), barley	2.14	1.68
10	H	Wheat, sweet clover (hay), corn, barley	2.14	1.60
11	I	Corn, barley, sweet clover (hay), wheat	2.11	1.61
12	J	Corn, wheat, fallow, barley	2.00	1.58
13	K	Corn, wheat, wheat, barley	1.94	1.70
14	K_x	Corn, wheat, wheat, barley	1.82	1.62
15	L	Wheat, peas, oats, sunflowers	2.00	1.62
16	M	Wheat, peas, oats, potatoes	2.07	1.61
17	N	Wheat, peas, oats, fallow	2.00	1.63
18	O	Wheat, sweet clover (hay), oats, sunflowers	1.90	1.63
19	P	Wheat, sweet clover (hay), oats, potatoes	2.00	1.61
20	Q	Wheat, sweet clover (hay), oats, fallow	1.94	1.65
		Average	2.00	1.64
		Max.	2.14	1.71
		Min.	1.82	1.58
		Total spread	.32	.13

Note: Ex, Fx, and Kx = wheat after wheat.

It will also be noted that the average gasoline color value of all 20 plots in 1930 was 2.00, and in 1931, 1.64. The seasonal variation, therefore, was greater than the variation due to cropping systems.

In 1930, the variation of 13 of the 20 samples was within 0.10 ± of the average gasoline color value. In 1931, all of the 20 plots were within 0.10 ± of the average gasoline color value, while 17 of 20 samples were within 0.05 ± of the average.

These data would indicate that the variation in pigmentation due to cropping systems is of minor importance and other factors must be present which contribute to the variations noted in the pigmentation of the same variety.

Regional Variation in Pigmentation

Table II shows a four-year average of gasoline color values for four varieties of durum wheat grown at Fargo, Dickinson, and Langdon. The average gasoline color values of durum wheats produced at Fargo and Dickinson do not show significant differences, but the Langdon values are distinctly higher.

TABLE II

REGIONAL VARIATION OF DURUMS IN GASOLINE COLOR VALUE

Variety	Gasoline Color values—4 year average 1928–1931 inclusive		
	Grown at Fargo	Grown at Dickinson	Grown at Langdon
Mindum	1.45	1.52	1.81
Kubanka	1.50	1.55	1.90
Nodak	1.28	1.26	1.56
Monad	1.06	1.00	1.34

It is hardly possible to ascribe this regional difference to a single factor. For the three locations, Fargo, Dickinson, and Langdon, the difference might be due to a combination of several factors. Some differences in environment of the three locations are noted below:

Elevation—Fargo, 935 feet above sea-level; Dickinson, 2543 feet; and Langdon, 1615 feet.

Soil [2]—Fargo, heavy black clay; Dickinson, Morton very fine sandy clay; Langdon, Barnes fine, sandy loam.

Average annual rainfall, inches—Fargo, 24.92; Dickinson, 15.41; and Langdon, 18.79.

Temperature of growing season—Langdon, significantly cooler than Fargo or Dickinson.

Langdon is intermediately between Fargo and Dickinson in elevation and rainfall, but the growing season is cooler at Langdon. One result of this combination of factors is a longer growing season at Langdon.[3] The days required between heading and maturity for the Kubanka variety are shown in Table III. The interval between

TABLE III

VARIATION IN TIME BETWEEN HEADING AND RIPENESS OF KUBANKA DURUM

Year	Days between heading and ripeness		
	Fargo	Dickinson	Langdon
1925	44	26	—
1926	37	21	—
1927	45	31	—
1928	46	36	52
1929	39	24	42
1930	35	21	39
1931	28	25	44

heading and maturity was longest at Langdon and shortest at Dickinson. The durum wheats produced in the Langdon region, therefore, have a longer interval for the most important period in the development of the wheat plant.

[2] From H. L. Walster, unpublished data.
[3] From T. E. Stoa, unpublished data.

Regional variation of significance in pigmentation of durum wheats does exist. This variation is probably due to the combined effect of several factors, including particularly temperature and rainfall.

Seasonal Variation in Pigmentation

Data on gasoline color of varieties was available for seven crop years at Fargo and Dickinson. Table IV shows the average gasoline color value of the Mindum and Kubanka varieties over a period of seven years and other climatic data.

TABLE IV

SEASONAL VARIATION IN GASOLINE COLOR VALUE AND CLIMATIC CONDITIONS

Year	Gasoline color value average Kubanka and Mindum varieties	Rainfall [1] during growing period (inches)	Mean May temperature	Mean June temperature	Mean July temperature
		Fargo			
1925	1.99	13.95	53.2	64.4	68.2
1926	1.63	7.63	60.2	60.2	70.2
1927	1.93	10.17	49.9	62.7	66.0
1928	1.49 [2]	11.85	59.1	60.2	69.3
1929	1.54	3.42	50.6	64.0	71.5
1930	1.39	8.01	53.4	65.0	73.6
1931	1.48	8.26	53.2	69.0	72.5
		Dickinson			
1925	1.58	7.75	53.9	61.4	67.6
1926	1.62	6.02	58.2	60.8	70.0
1927	2.07	12.31	48.0	61.4	65.4
1928	1.60 [2]	10.43	58.4	57.1	66.8
1929	1.53	7.32	47.7	59.4	70.2
1930	1.33	5.89	48.8	60.8	71.6
1931	1.68	6.32	52.8	67.8	69.2

[1] Actual inches of rainfall between date of seeding and date of harvest.
[2] Heavy rains immediately after cutting damaged the grain and injured color.

At Fargo, high gasoline color values were noted in 1925 and 1927. It will also be noted that the 1928 season rainfall was comparatively high, but the gasoline color values were low. The low gasoline color value in 1928, however, was due to damage of the grain by heavy rains immediately following harvest. At Fargo, the mean July temperatures for 1925 and 1927 were the lowest of the seven years.

At Dickinson, a high average gasoline color value was found only in 1927. The low gasoline color value in 1928 at Dickinson, as at Fargo, was due to damage following harvest. The rainfall in 1927 was significantly higher at Dickinson, as compared to other years, with the exception of 1928. The July, 1927 temperature was the lowest for the seven year period, and the May temperature was also comparatively low.

The seasonal variation in degree of pigmentation is, therefore, due in part at least to variation in rainfall and temperature. Ample rainfall and low mean temperatures during the growing season are conditions which tend to produce good yields, and the same conditions evidently tend to produce durum crops with a high degree of pigmentation. Hot, dry seasons produce durum wheat of relatively poorer color and also reduce the yield.

Summary

1. Cropping systems and preceding crops are minor factors in affecting the color of durum wheats.

2. A significant regional variation in pigmentation of durum wheats has been noted. In North Dakota, the Langdon area tends to produce better colored durum wheat than either the Fargo or Dickinson area.

3. A significant seasonal variation in color has been noted. Cool seasons, with ample rainfall, produce durum wheats of relatively better color than the hot, dry seasons.

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North Dakota Expt. Sta. Cir. 46.

INVESTIGATION OF A MERCURY VAPOR LAMP FOR ACCELERATING THE BLEACHING ACTION OF DIBENZOYL PEROXIDE

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Introduction

It is well known that flour treated with dibenzoyl peroxide (Novadel reagent) is not bleached immediately but requires a fairly long time interval to complete the reaction. In this respect, dibenzoyl peroxide differs from gaseous bleaching agents which act almost immediately. A study of the rate at which several bleaching agents react has been reported by Ferrari and Bailey (1929a). They found that dibenzoyl peroxide required from 20 to 40 hours to reach the maximum effect, depending on the original concentration of carotinoid pigments in the flour and the quantity of the bleaching reagent used. The larger quantities of dibenzoyl peroxide give a more vigorous initial bleaching.

In the manufacture of flour, it is frequently desirable to immediately ascertain the potential bleaching effect of dibenzoyl peroxide. It has been known for some time that the reaction rate can be accelerated by the use of radiant energy of comparatively short wave lengths. A claim to this effect is made in the original patents covering the use of dibenzoyl peroxide as a flour bleaching agent (Sutherland, 1921, 1925; Gelissen, 1924). A laboratory device for accelerating this reaction with radiant energy from a mercury vapor lamp has been designed by the Wallace and Tiernan Co., Inc., who kindly loaned it for this study.

Description of Mercury Vapor Lamp Unit

The mercury vapor lamp used is an adaptation of a General Electric Cooper-Hewitt Junior lamp. A diagram of the lamp construction is given in Figure 1, and a photograph of the equipment is illustrated in Figure 2. The lamp is suspended from cadmium plated arms that are attached to a heavy wooden base. An automatic timing device permits accurate control of the irradiation process. A wooden tray is mounted on an angle under the electrode vessel in such a way that the exposed layer of flour is parallel with and 3.2 cm. distant from the tube. The wooden tray 12 × 61 cm. contains a shallow trough 1 mm. deep, and exposes a surface of flour approximately

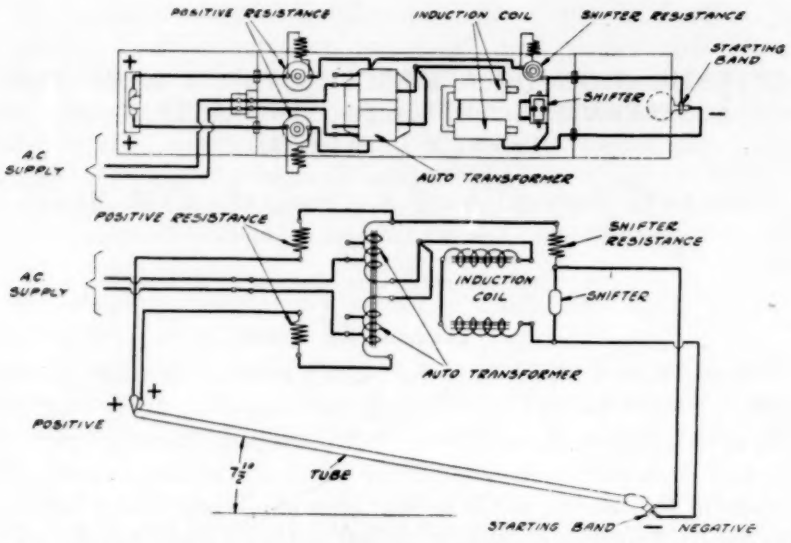


Fig. 1. Wiring diagram of mercury vapor bleaching accelerator. (Supplied by General Electric Vapor Lamp Co., Hoboken, N. J.)

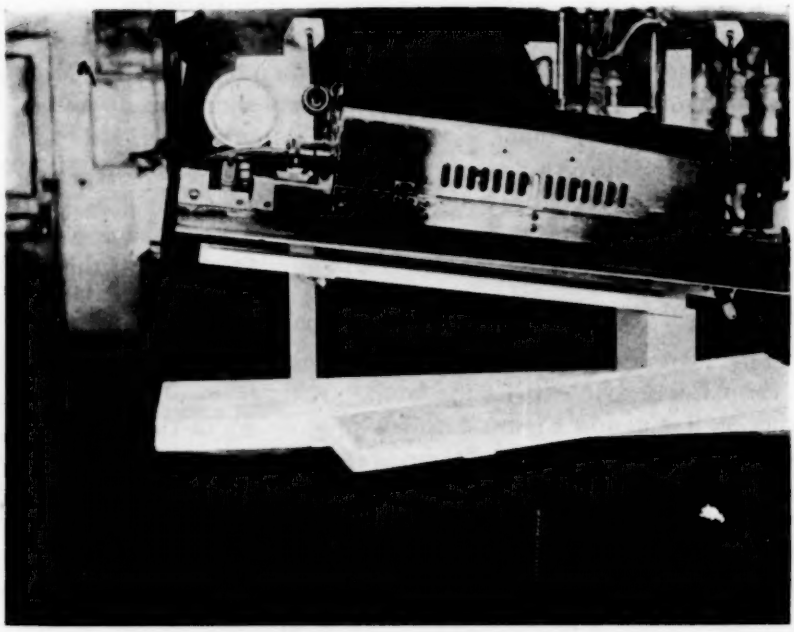


Fig. 2. Mercury vapor lamp unit used in irradiation studies.

10 × 60 cm. The flour is placed loosely on the tray and leveled with a straight edge, without compressing. A rim on the tray 1 cm. wide guides the straight edge and insures an even depth of flour.

The electrode vessel is Uviol glass, which is reported by the manufacturer to transmit 50% of the radiant energy at 280 mμ. and 10% at 258 mμ. Thus, a considerable amount of ultra-violet radiant energy is transmitted. While quartz would transmit more efficiently, the data included here will show that Uviol glass performs satisfactorily for this purpose.

Experimental Procedure

In preparing samples treated with dibenzoyl peroxide (Novadel reagent) in the laboratory, the requisite quantity of the bleaching agent was weighed on an analytical balance and added to one kilogram of flour contained in a small, motor-driven, Miracle batch mixer. Previous experience with this mixer demonstrated its high efficiency for this type of mixing. The mixing operation was timed 10 minutes. As soon as mixing was completed, the flour was spread on the tray and placed under the mercury vapor lamp which had been operating at least 10 minutes to bring it to full efficiency. The Novadel reagent used in these experiments contained 15.8% of dibenzoyl peroxide determined by titration with sodium thiosulphate solution.

At the end of the irradiation period, 20 gms. of flour were weighed out in a wide-mouthed, glass-stoppered bottle and 100 cc. of a mixture of cleaner's naphtha and absolute alcohol were added. The ratio of cleaner's naphtha to alcohol was 93 to 7. This mixed solvent has been used as part of the standard procedure for estimating carotinoid pigments quantitatively in this laboratory. It removes more pigment than cleaner's naphtha alone. After thorough agitation, the sample and solvent were allowed to stand overnight, taking due precautions to shield the bottles from light and shaking occasionally. The following day agitation of the sample was repeated and the flour allowed to settle. The supernatant liquid was decanted into a centrifuge tube, covered with rubber dam, and centrifuged at high speed for not less than 20 minutes. The clear liquid was siphoned from the tube by means of a capillary siphon bent back upon itself in the form of the letter U. Transmittancy of the extract was determined in a 9 cm. cell with a Koenig-Martens spectrophotometer at a wave length of 435.8 mμ. using a mercury vapor light source, and the transmittancy values converted into carotin concentration according to the procedure described by Ferrari and Bailey (1929b, c). The Koenig-Martens spectrophotometer and its operation have been described by McNicholas (1928). The two compartment cell in which transmittancy

of the extracts was determined was designed several years ago by the senior author and has been found very convenient.

Experimental Results

Flour sample No. 993 was mixed with the Novadel reagent at the rate of 7, 14, and 21 gms. per barrel of flour, and irradiated for various time intervals up to 60 minutes. Aliquots of the Novadel treated flour were set aside for comparison with irradiated samples at the end of each irradiation period. It was necessary, of course, to prepare separate aliquots of flour treated with dibenzoyl peroxide for each of the irradiation treatments in order to subject freshly treated flour to the irradiation process in each instance. At the end of each treatment, the flour was weighed promptly and the solvent added. The concentration of carotin in the flour was determined in the manner described. The detailed results are recorded in Table I and are shown graphically in Figure 3.

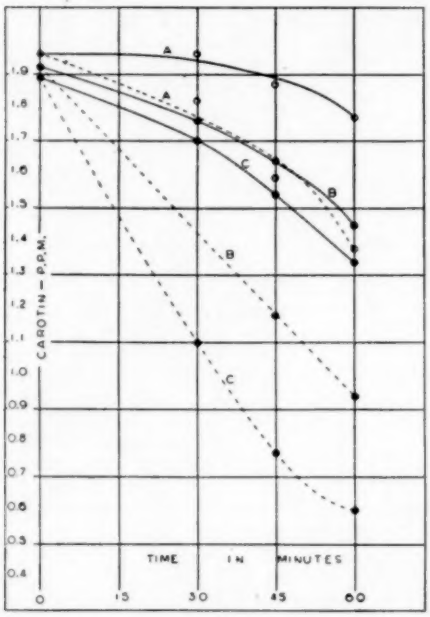


Fig. 3. Comparison of irradiated and non-irradiated flour No. 993 bleached with the Novadel reagent at the rate of 7, 14 and 21 gms. (Data in Table I.)

Solid lines = not irradiated.
Broken lines = irradiated.

A. Bleached with 7 gms. Novadel reagent.
B. Bleached with 14 gms. Novadel reagent.
C. Bleached with 21 gms. Novadel reagent.

Carotin in unbleached flour 2.03 p.p.m.

The untreated flour had a carotin concentration of 2.03 parts per million. At the end of 48 hours, flour treated with 7, 14 and 21 gms. of Novadel per barrel (not irradiated) had a carotin concentration of

1.54, 1.03 and 0.72 p.p.m. of flour respectively. These latter figures, then, represent the carotin concentrations it was desired to attain in a short time interval by means of the irradiation treatment. It appears that 50 minutes irradiation, on the average, accomplished the desired results in this experiment for all three bleaching treatments.

A thermometer was placed at each end of the tray with its bulb just touching the flour surface under the mercury vapor lamp. The maximum average temperature reached is recorded in Table I. The

TABLE I

COMPARISON OF IRRADIATED AND NON-IRRADIATED FLOUR NO. 993 BLEACHED WITH THE NOVADEL REAGENT AT THE RATE OF 7, 14 AND 21 GMS. PER BARREL OF FLOUR

Not irradiated		Irradiated		
Time after bleaching	Carotin, p.p.m. of flour	Time irradiated	Temperature at flour surface C°	Carotin, p.p.m. of flour
Sample treated with 7 gms. of Novadel reagent per barrel of flour				
Immediately	1.96			
30 minutes	1.96	30 minutes	39	1.82
45 minutes	1.87	45 minutes	43	1.59
60 minutes	1.77	60 minutes	45	1.38
48 hours	1.54			
Sample treated with 14 gms. of Novadel reagent per barrel of flour				
Immediately	1.92			
30 minutes	1.76	30 minutes	36	1.75
45 minutes	1.64	45 minutes	40	1.18
60 minutes	1.45	60 minutes	45	0.94
48 hours	1.03			
Sample treated with 21 gms. of Novadel reagent per barrel of flour				
Immediately	1.89			
30 minutes	1.70	30 minutes	43	1.10
45 minutes	1.54	45 minutes	44	0.77
60 minutes	1.34	60 minutes	—	0.60
48 hours	0.72			
Unbleached flour	2.03			

temperature rise was gradual up to the maximum and a longer treatment would probably have effected a further increase. The maximum noted was 45° C., attained after 60 minutes irradiation.

This preliminary experiment suggested the advisability of determining the moisture content of the flour after irradiation. If the flour were dried out appreciably during this process, it would affect the results of the determination of carotin on a 20 gm. sample. Accordingly, moisture determinations were made by the air oven method at 130° C. A tabulation of the data is given in Table II. A flour with an initial moisture content of 12.6% decreased gradually to 4.6% at

TABLE II

EFFECT OF IRRADIATING FLOUR FOR VARIOUS TIME INTERVALS ON THE MOISTURE CONTENT OF THE FLOUR. MOISTURE WAS DETERMINED IN AN AIR OVEN AT 130° C. HEATING FOR ONE HOUR

Irradiation time	Moisture
Minutes	P.ct.
0	12.6
30	7.7
40	6.9
45	6.7
50	6.4
60	6.0
70	5.6
80	5.2
90	4.8
100	4.6

the end of 100 minutes irradiation. Using these figures as a basis, a curve was drawn using time and per cent moisture as coordinates. This curve is given in Figure 4. On the basis of this curve, all determi-

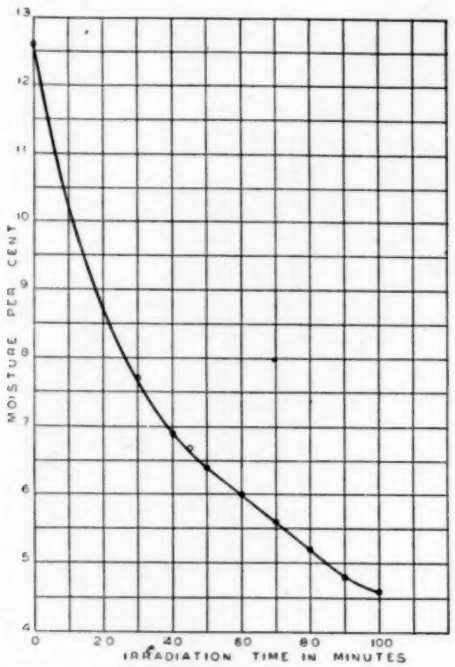


Fig. 4. Relation between irradiation time and moisture content of flour.

nations of carotin subsequently made were corrected for moisture, and the results are expressed uniformly on the basis of 13.5% of moisture.

As the moisture of flour falls rapidly on irradiation with the equipment described, it will be necessary for those desiring to use the

Pekar test for color comparisons to take this fact into account. Alcock and Ediger (1929) showed that the moisture of flour influenced the Pekar test to a marked degree, the drier flours appearing lighter in color whether the test was made wet or dry.

It is natural to inquire about the bleaching of flour due to the irradiation treatment alone. Ferrari and Bailey (1929a) studied the bleaching effect of ultra-violet radiant energy emitted by quartz mercury-vapor arc lamps. An appreciable bleaching was noticed, but the action was slow. Thus, in 7 hours the strongest source of ultra-violet radiant energy that was employed bleached 47% of the carotin originally present in the flour in 7 hours. A weaker source of radiant energy bleached from 2.5% to 10% in 5 hours. The electrode vessel in the bleaching accelerator was constructed of Uviol glass, which transmits ultra-violet radiant energy much less efficiently than quartz. On the other hand, the electrode vessel was very much larger than the quartz electrodes used in the investigation to which reference was made above. It appeared desirable to measure the effect of irradiation alone with the present equipment, and this was done. Samples of flour were irradiated for various time intervals up to 60 minutes. A slight decrease in the concentration of carotin could be detected, but it was of small enough magnitude (several hundredths) to justify eliminating it from consideration in the present problem.

Another question of importance in the examination of flour extracts, where the sample has been bleached with dibenzoyl peroxide, concerns whether or not bleaching ceases when the solvent is added to the flour. Dibenzoyl peroxide is soluble to some extent in cleaner's naphtha containing alcohol, and it might conceivably act on carotin in solution during the extraction period. In the experience of the authors, bleaching ceases upon the addition of the solvent to the flour in so far as the spectrophotometric procedure is able to detect it. It may be of interest to include some data to substantiate this statement, as none have ever been published. The data are given in Table III. Extracts of flour treated with the Novadel reagent both irradiated and not irradiated were examined in the conventional manner. Solvent was added before the Novadel reagent completely bleached the sample. Forty-eight hours after the customary examination, the extracts were again examined spectrophotometrically. The average change in carotin concentration was 0.03 p.p.m., no more than an extract from an unbleached flour would change on standing the equivalent length of time, and not much more than the experimental error of the determination.

A series of irradiation experiments were run under carefully controlled conditions, using flours having widely different carotin

TABLE III

COMPARISON OF THE CAROTIN CONCENTRATIONS OF FLOUR EXTRACTS FROM DIBENZOYL PEROXIDE TREATED FLOURS EXAMINED 48 HOURS LATER TO DETERMINE WHETHER OR NOT THE PEROXIDE BLEACHED IN SOLUTION. FLOUR NO. 1192 TREATED WITH 11 GMS. OF NOVADEL. SOLVENT, CLEANER'S NAPHTHA-ALCOHOL. RESULTS ON BASIS OF 13.5% MOISTURE IN THE FLOUR.

Sample description	Carotin, p.p.m. of flour		
	Customary time interval	Same extract 48 hours later	Difference
Unbleached flour	2.12		
Completely bleached flour	1.17		
Bleached flour, sampled immediately after bleaching	2.07	1.99	0.08
Bleached flour, sampled immediately after bleaching	2.04	2.00	0.04
Bleached flour, irradiated 30 minutes, then sampled	1.53	1.51	0.02
Bleached flour, irradiated 40 minutes, then sampled	1.30	1.21	0.09
Bleached flour, irradiated 50 minutes, then sampled	1.12	1.09	0.03
Bleached flour, irradiated 60 minutes, then sampled	1.13	1.13	0.00
Bleached flour, irradiated 70 minutes, then sampled	1.04	1.03	0.01
Not irradiated, sampled after 60 minutes	1.90	1.89	0.01
Not irradiated, sampled after 60 minutes	1.89	1.89	0.00
Average difference			0.03

concentrations, and treating them at the rate of 11 and 23 gms. of Novadel per barrel of flour. Irradiation was timed in some cases as long as 100 minutes. Some representative data illustrating the results obtained are given in Tables IV, V, and VI.

TABLE IV

COMPARISON OF IRRADIATED AND NON-IRRADIATED FLOUR NO. 1193 BLEACHED WITH THE NOVADEL REAGENT AT THE RATE OF 11 AND 23 GMS. PER BARREL OF FLOUR. RESULTS ON BASIS OF 13.5% MOISTURE IN THE FLOUR

Sample treated with 23 gms. of Novadel reagent per barrel of flour				
Not irradiated		Irradiated		Flour 48 hours later
Time after bleaching	Carotin, p.p.m. of flour	Time irradiated	Carotin, p.p.m. of flour	
Immediately	2.05			
30 minutes	1.83	30 minutes	1.12	
40 minutes	1.79	40 minutes	1.04	
50 minutes	1.75	50 minutes	0.77	
60 minutes	1.65	60 minutes	0.64	
		70 minutes	0.64	
		90 minutes	0.64	
		100 minutes	0.63	
120 minutes	1.40			
150 minutes	1.33			
48 hours	0.60			
Unbleached flour	2.24			
Sample treated with 11 gms. Novadel reagent per barrel of flour				
Immediately	2.13	30 minutes	1.59	0.95
48 hours	1.17	40 minutes	1.39	0.95
		50 minutes	1.12	
		60 minutes	1.08	
		70 minutes	1.01	

TABLE V

COMPARISON OF IRRADIATED AND NON-IRRADIATED FLOUR NO. 1191 BLEACHED WITH THE NOVADEL REAGENT AT THE RATE OF 11 AND 23 GMS. PER BARREL OF FLOUR. RESULTS ON BASIS OF 13.5% MOISTURE IN THE FLOUR

Sample treated with 11 gms. of Novadel reagent per barrel of flour				
Not irradiated		Irradiated		
Time after bleaching	Carotin p.p.m. of flour	Time irradiated	Carotin p.p.m. of flour	Flour 48 hours later
Immediately	1.25	30 minutes	0.91	0.52
48 hours	0.63	40 minutes	0.76	0.53
		50 minutes	0.67	0.50
		60 minutes	0.65	0.48
		70 minutes	0.63	
Unbleached flour	1.28			
Sample treated with 23 gms. of Novadel reagent per barrel of flour				
Immediately	1.18			
30 minutes	1.07	30 minutes	0.80	
40 minutes	1.05	40 minutes	0.61	
48 hours	0.45	60 minutes	0.44	

TABLE VI

COMPARISON OF IRRADIATED AND NON-IRRADIATED FLOUR NO. 1194 BLEACHED WITH THE NOVADEL REAGENT AT THE RATE OF 23 GMS. PER BARREL OF FLOUR. RESULTS ON BASIS OF 13.5% MOISTURE IN THE FLOUR

Not irradiated		Irradiated		
Time after bleaching	Carotin p.p.m. of flour	Time irradiated	Carotin p.p.m. of flour	Flour 48 hours later
Immediately	1.18			
30 minutes	1.08	30 minutes	0.66	0.29
40 minutes	1.12	40 minutes	0.58	——
50 minutes	1.05	50 minutes	0.50	0.32
60 minutes	1.02	60 minutes	0.52	0.30
70 minutes	0.98	70 minutes	0.52	0.31
80 minutes	0.95	80 minutes	0.46	0.27
90 minutes	0.93	90 minutes	0.45	0.33
100 minutes	0.90	100 minutes	0.42	——
110 minutes	0.87			
120 minutes	0.84			
130 minutes	0.82			
24 hours	0.36			
Unbleached flour	1.34			

Flour No. 1193 (see Table IV) had an initial carotin concentration of 2.24 p.p.m. At the end of 48 hours, 23 gms. of Novadel reduced the concentration to 0.60 p.p.m. Irradiation of the mixture of flour and Novadel reagent for 60 minutes reduced the concentration to substantially the same figure. It is interesting to note that longer irradiation periods up to 100 minutes failed to reduce the carotin concentration further. Apparently the reaction of the dibenzoyl peroxide was essentially complete at the end of 60 minutes and irradiation

beyond this time contributed a slight bleaching action, perhaps due to the radiant energy alone. By way of comparison, the carotin concentration of non-irradiated flours is given in the table, and the slowness with which bleaching normally occurs is illustrated. The data are depicted graphically in Figure 5.

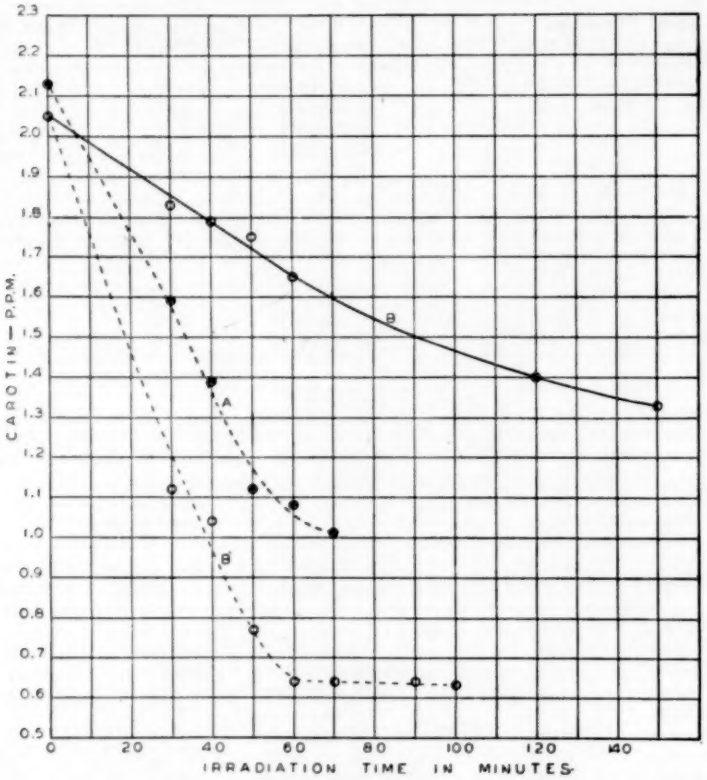


Fig. 5. Comparison of irradiated and non-irradiated flour No. 1193 bleached with the Novadel reagent at the rate of 11 and 23 gms. per barrel of flour. (Data in Table IV.)

Solid lines = not irradiated. A. Bleached with 11 gms. Novadel reagent.
Broken lines = irradiated. B. Bleached with 23 gms. Novadel reagent.

Carotin in unbleached flour 2.24 p.p.m.

The same sample (flour No. 1193—Table IV) treated with 11 gms. of Novadel per barrel of flour reached a carotin concentration of 1.17 p.p.m. after 48 hours. Irradiation of the treated sample for a period in the range of 50 minutes accomplished the same bleaching effect.

A similar experiment was carried out with flour No. 1191 (see Table V and Figure 6). This flour was previously treated with Agene which reduced its carotin concentration to 1.28 p.p.m. before the addition of Novadel. Treated with Novadel at the rate of 11 gms. per barrel of flour, the carotin concentration dropped to 0.63 p.p.m. at the

end of 48 hours. Irradiation of the treated sample for 60 to 70 minutes accomplished the same bleaching effect as standing for 48 hours. It will be noticed that the change in concentration after irradiating 50 minutes was very slight. A similar effect was pointed out in discussing the results of the previous experiment. Novadel at the rate of 23 gms. per barrel reduced the carotin concentration to 0.45 p.p.m. at the end of 48 hours, while irradiation of the Novadel treated flour for 60 minutes accomplished the same purpose.

The data in Table VI are included to show what happens when it is desired to bleach a sample of flour heavily to reduce its carotin concentration to a very low level. The flour (No. 1194) contained initially 1.34 parts of carotin per million parts of flour after treatment with Agene and was subsequently treated with 23 gms. of Novadel. In 2 hours, the carotin concentration fell to a fairly low level (0.84 p.p.m.), and at the end of 24 hours it had fallen to 0.36 p.p.m. The latter figure represents a much smaller quantity of carotin than most well bleached flours contain. Irradiation for as little as 30 minutes reduced the carotin concentration to 0.66 p.p.m. of flour. Irradiation for longer time intervals did not reduce the pigmentation proportionately. On the contrary, the bleaching acceleration was markedly slower and, even after 100 minutes irradiation, the carotin concentration had not quite reached the level of the flour that stood for 24 hours. In instances where the quantity of Novadel reagent is so greatly in excess of the requirements, no doubt much of the bleaching effect is wasted. It would appear from the data on the irradiated samples that it is possible to accelerate the reaction to a point where the efficiency of the reagent is impaired. The data are shown graphically in Figure 6.

Acceleration of the decomposition of dibenzoyl peroxide in flour by ultra-violet radiant energy is an interesting reaction. In patents covering the use of dibenzoyl peroxide as a flour bleaching agent, Sutherland (1921, 1925) and Gelissen (1924) mention the use of actinic light and specifically ultra-violet radiant energy as a means for activating the process. Brodie (1863) investigated the decomposition of dibenzoyl peroxide by itself, and found that on heating it decomposed with an explosion. Mixed with sand, it decomposed smoothly at 85° C., yielding one molecule of carbon dioxide for each molecule of peroxide, and a residue whose nature could not be determined. Gelissen and Hermans (1925a) confirmed these results. They found, in addition, that by mixing dibenzoyl peroxide with a catalyst (whose nature they did not divulge) and carefully warming, two equivalents of carbon dioxide were evolved. Distillation of the residue with steam yielded diphenyl. Due to the consistent appearance of *p*-phenylbenzoic acid in many of the reactions of dibenzoyl peroxide, Gelissen

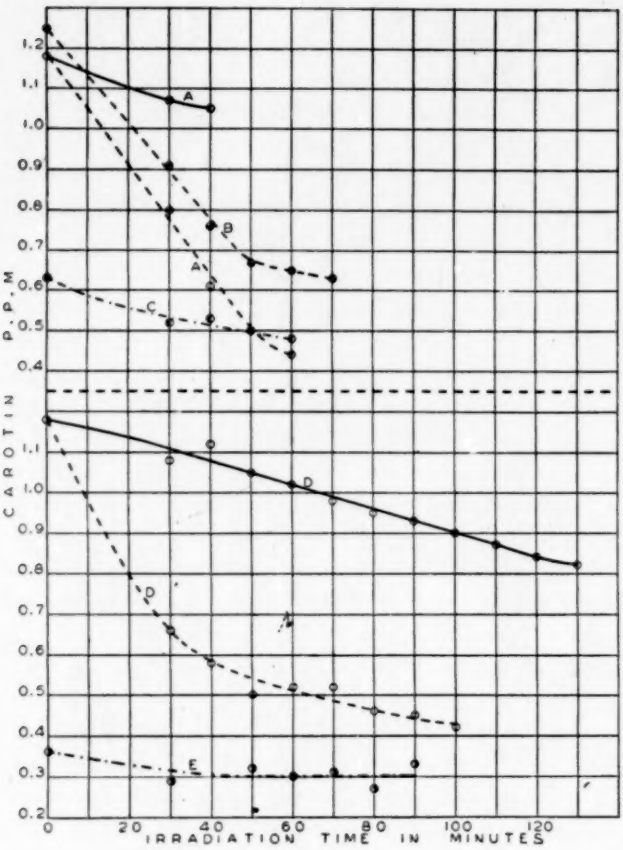
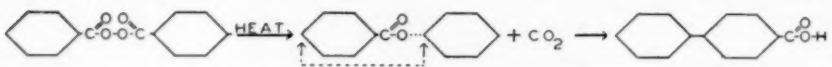


Fig. 6. Comparison of irradiated and non-irradiated flours bleached with the Novadel reagent at the rate of 11 and 23 gms. per barrel of flour. (Data in Table V and VI.)

Solid lines = not irradiated.
Broken lines = irradiated.

A. Flour No. 1191 bleached with 23 gms. Novadel per barrel of flour.
B. Flour No. 1191 bleached with 11 gms. Novadel per barrel of flour.
C. Irradiated flour No. 1191 (Curve B) 48 hours after irradiation.
D. Flour No. 1194 bleached with 23 gms. Novadel per barrel of flour.
E. Irradiated flour No. 1194 (Curve D) 48 hours after irradiation.

and Hermans (1925b) believe that it is formed in the thermal decomposition of dibenzoyl peroxide. The reaction may be written:



The reaction when dibenzoyl peroxide is gently heated with a catalyst may be written:

Thus, the reaction products depend upon the conditions under which decomposition takes place.

Fichter and Schnider (1930) investigated the decomposition of dibenzoyl peroxide under the influence of ultra-violet radiant energy. They showed that carbon dioxide and diphenyl were the chief reaction products along with a resin that was not accurately characterized. Irradiation with dibenzoyl peroxide in solvents also yielded different reaction products than did heat treatment. Irradiation of dry dibenzoyl peroxide with ultra-violet radiant energy yielded 70% of the theoretical amount of diphenyl, while heat yielded 53%. Ultra-violet radiant energy appears to give the same reaction as a catalyst plus gentle heat, rather than heat alone.

The decomposition products of dibenzoyl peroxide in flour can only be speculated upon. Irradiated flour never attained a temperature in excess of 50° C. in our experiments, but since the temperature was much higher than ordinary, it, as well as the radiant energy, may have some influence on the course of the reaction. It seems clear that carbon dioxide and diphenyl will form along with other decomposition products whose nature it is impossible to state with certainty at this time. Possibly the reaction products formed through the use of actinic radiant energy may be more effective bleaching agents than dibenzoyl peroxide itself.

Attention is invited to the columns in Tables IV, V, and VI headed "Irradiated—Flour 48 hours later." After the irradiation periods indicated, the flours were also sampled after 48 hours standing. It will be noticed that the irradiated flours that stood for 48 hours invariably reached a lower level of carotin concentration than samples which stood for the same period of time but were not irradiated. The increased bleaching effect can not be attributed to differences in the moisture content of the flour, because all results have been expressed on the basis of 13.5% moisture. Neither can the increased bleaching effect be ascribed to the radiant energy alone, because this effect was measured and was found to be practically insignificant. The reason is obscure and probably is related to a more efficient utilization of the potential bleaching power of the added dibenzoyl peroxide. The reaction of dibenzoyl peroxide is activated by the impinging radiant energy increasing its reaction rate to a certain higher level and maintaining this increased rate until the peroxide has been exhausted. Under these circumstances, dibenzoyl peroxide may conceivably be a more efficient bleaching agent. This reasoning is not inconsistent with the fact that the potential oxidizing power of the added peroxide is many times that required for the oxidation of the carotin present. A number of years ago, the manufacturer of Novadel was able to reduce the quantity of dibenzoyl peroxide in the reagent from 25% to 16% by its more efficient utilization.

Other experiments similar to those described were carried out and yielded similar results. It is believed that sufficient data have been included to illustrate the essential facts.

Summary

A laboratory device for accelerating the bleaching rate of dibenzoyl peroxide (the Novadel reagent) is described. The acceleration is accomplished by means of radiant energy from a mercury vapor arc. The purpose of this equipment is to duplicate in a comparatively short time the bleaching effect of dibenzoyl peroxide that has stood in contact with flour for 48 hours. The mill laboratory would thus be able to determine the extent of their bleaching treatment with this reagent without waiting the customary 24 to 48 hours for it to take place.

Flour sampies bleached with dibenzoyl peroxide and irradiated for definite time intervals are investigated for their carotinoid pigment concentration using the spectrophotometric procedure. Results are expressed in parts of carotin per million parts of flour. Certain details of this method different from those previously published are given. The extracts are examined in a special cell and the transmittancy of the extracts are determined using a Koenig-Martens spectrophotometer and a mercury vapor light source.

Upon irradiation with the bleaching accelerator, flours rapidly lose moisture. An initial moisture content of 12.6% decreased to 4.6% after irradiation up to 100 minutes. For this reason, all data are corrected for moisture and expressed on a common moisture basis of 13.5%.

Flours with an initial carotin concentration in the range of 2.00 p.p.m. require at least 50 minutes irradiation after treatment with 11 gms. of Novadel per barrel of flour to reduce the concentration of the carotinoid pigments to the level reached upon standing for 48 hours. Similarly, 60 minutes irradiation are required when 23 gms. Novadel per barrel of flour are used. Flours having smaller initial concentrations of pigment (in the range of 1.3 p.p.m.) require 60 minutes irradiation after treatment with 11 gms. Novadel per barrel of flour. When the reduction of carotin concentrations to much lower levels is required—below say 0.5 p.p.m.—a longer irradiation period is necessary. This follows as a natural consequence of the fact that, when the carotinoid pigments are removed by bleaching, as the quantity remaining gets smaller it becomes increasingly difficult to remove by bleaching.

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THE GUMS OF THE CEREAL GRAINS [1]

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Introduction

Throughout the literature dealing with the protein fractions of cereal grains, there have been numerous references to the gummy substances that often cause some inconvenience in the separation of the various protein fractions. Besides a few early descriptions of these gums, no serious investigations seem to have been recorded.

The problem was reopened by Hoffman and Gortner (1927). These workers attempted to isolate globulin, albumin, and proteose fractions from wheat flour according to methods described by Osborne (1907). Instead of a proteose fraction, they obtained a substance that contained only a small amount of nitrogen which when dissolved in water formed an extremely gummy, viscous solution. Since no detailed study of such material has been recorded, the present work on the isolation and chemical nature of the material was undertaken.

Historical

A review of the literature has revealed two methods for isolating gums from cereal products, yielding in general two different types of gums: (1) Extraction of the flour or meal with water or dilute neutral salt solutions, (2) extraction by dilute sodium hydroxide. The latter method has been used extensively with many different types of plant materials and yields a product that has been designated by many authors as "hemicellulose."

Von Bibra (1861) and Ritthausen (1866) isolated a gum from rye flour by extraction with 10% sodium chloride. Ritthausen (1867) indicated that the gums were polymerized hexose sugars. Effront (1897) described amorphous gummy substances from cereal grains, which Ritthausen (1897) claimed were identical with his earlier preparations. Haas and Hill (1928) have suggested that these gums were polymers of mannose. Osborne (1894, 1895) has noted the necessary and troublesome separation of gums in isolating protein fractions ex-

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tracted by dilute salt solutions from rye and barley meals. Hoffman and Gortner (1927) extracted wheat flour with 5% potassium sulfate, and after the separation of the protein fractions, they obtained a gum which was shown to be a polymerized carbohydrate. Gums obtained by this method have received little attention, probably because the protein fractions have been the object of primary interest.

The gums obtained by extracting cereal grains with dilute sodium hydroxide have been described in many papers on hemicelluloses. Gudkow (1870) extracted wheat bran with dilute potassium hydroxide and obtained a gummy substance containing large amounts of pentose sugar. Stone and Tollens (1888) confirmed this report and isolated the phenylosazone of arabinose. Steiger and Schulze (1890) isolated arabinose from a white amorphous gum, metaraban. In a later paper, Schulze (1892) reviewed extensive investigations of similar gums, in particular, those of wheat and rye bran. Schulze (1891) defined the hemicelluloses as substances "extracted from plant materials by dilute alkali and easily hydrolyzed by hot dilute mineral acids." This definition has undergone some modification in recent years. At present two classes of hemicelluloses are distinguished: (1) The acidic hemicellulose (polyuronide) characterized by a relatively low content of hexose uronic acids, (2) the polysaccharide hemicellulose, containing no uronic acids. Candlin and Schryver (1928), Norris and Preece (1930), and Anderson (1931) have strongly recommended this basis of classification. A number of papers point out a significant difference in the amount of hexose uronic acids found in the different types of gums, such as: pectins, mucilages, natural gums, bacterial gum hemicelluloses, etc. It has been suggested that, until we have more definite knowledge of the structures, the content of uronic acids might serve as a basis for chemical differentiation.

The significance of the uronic acids, which escaped the attention of the earlier investigators,[2] led Norris and Preece (1930) to reinvestigate the earlier work on wheat bran. Their crude product was separated into 4 fractions—two contained polymerized sugars and hexose uronic acids; and two contained only polymerized sugars, largely pentosans. It is unlikely that these hemicelluloses containing uronic acids were impure pectin fractions because Nanji and Norman (1928) indicated only traces of pectic materials in cereal grains.

Clayson and Schryver (1923) obtained a hemicellulose of different nature by extracting wheat flour with dilute sodium hydroxide. They obtained a sugar polymer yielding only glucose on hydrolysis. Larmour (1927) has reported the isolation of gummy substances from

[2] The hexose uronic acids were not recognized until qualitative and quantitative methods were developed by Tollens (1908), Lefevre and Tollens (1907), Nanji, Paton, and Ling (1925), and Dickson, Otterson, and Link (1930).

alkaline extracts of several cereal grains. Subsequent examination [3] indicates that these gums may be similar to the hemicelluloses obtained by Clayson and Schryver, although Larmour's preparation did contain appreciable amounts of pentose sugars.

Experimental

PREPARATION OF GUMS. "Wheat gum—1927" was part of the sample prepared by Hoffman and Gortner (1927). This sample was light grey in color, hard lumpy material, and difficult to grind.

A similar sample prepared by identical technic from durum flour yielding "durum gum—1929, "was, however, light yellow in color. The yield was 12 gms. from 12 kilos of flour.

A second preparation, "durum gum—1930," was prepared as above but the purification modified as follows: Instead of dialyzing the purified solution in a cellophane bag against 95% ethanol as described by Hoffman and Gortner (1927), 95% ethanol was added until no further precipitation occurred. The precipitate was filtered with suction, washed with ethanol and ether and dried. The yield was 5 gms. from 12 kilos of flour. The product was pure white and easily crumbled to a fine white powder.

Twelve kilos of rye flour were used to prepare the sample of "rye gum—1930." In this case 1 kilo of flour was suspended in 7 liters of 5% potassium sulfate solution. After the separation of the protein fractions, the concentrated solution was saturated with ammonium sulfate. The crude gum suddenly separated in a voluminous tough spongy mass. A relatively large amount of crude gum was obtained but unfortunately, most of it was lost in the process of purification. The yield was 5 gms. of tough, brown, lumpy material.

Pearl barley was finely ground and the resulting meal was used for the extractions. Two attempts to prepare gums from barley meal failed and the procedure was modified as follows: 2.25 kilos of meal were extracted with approximately 10 liters of 5% potassium sulfate. The meal was extracted a second time and the combined extracts were evaporated under reduced pressure until a large amount of potassium sulfate and protein material had been precipitated. The concentrate was filtered yielding a water-clear filtrate which was again concentrated under reduced pressure. The crystallized potassium sulfate was removed by filtration and the clear filtrate was dialyzed until free from sulfates. The dialyzed solution was concentrated to a thick gummy sirup and 95% ethanol was added precipitating a white voluminous coagulum, which was filtered, washed with ethanol and ether, and dried with acetone. The crude gum was dispersed in water and

[3] *Vide infra.*

heated to boiling. Part of the material resisted solution and was removed by filtration. The water clear filtrate was evaporated to a sirup and treated with 500 cc. of 95% ethanol. The precipitate was filtered, washed with ethanol and ether, dried with acetone, and finally dried at 100° C. The yield was 4 gms. of an amber substance resembling gum arabic. The material on grinding formed a tan-colored powder.

The above methods were exceedingly long and tedious and since further studies were contemplated, a shorter and more efficient method seemed desirable. The following gave promise as a much easier method: 1.5 kilos of a clear wheat flour were suspended in 5.5 liters of half saturated ammonium sulfate solution. After standing with frequent stirring for several days at room temperature, the flour suspension settled and about one-half the total volume was siphoned off as a clear brownish liquid. Solid ammonium sulfate was then added to full saturation, whereupon a voluminous coagulum separated sharply. This precipitate was removed by filtration, dispersed in water, and dialyzed in a cellophane bag until free from sulfates. The dialysate was evaporated to a sirup under reduced pressure and poured into a liter of 95% ethanol, yielding a white voluminous precipitate. The crude precipitate was dispersed in water, heated to boiling to remove heat-coagulable proteins, and filtered with suction. The clear colorless filtrate was dialyzed against water to the absence of sulfates and then evaporated under reduced pressure to a sirup. The sirup was poured into 95% ethanol; and the white coagulum was filtered, washed with ethanol and ether, dried in acetone, and finally at 100° C. The yield was 4 gms. of a white crumbly material, easily ground to a fine white powder.

Analysis of the Gums.—The gums were analyzed for their most important constituents and the results are recorded in Table I. The

TABLE I

Analyses of the Cereal Gums

Constituent	Wheat 1927	Durum 1929	Durum 1930	Rye 1930	Wheat 1931	Barley 1931
	P.ct.	P.ct.	P.ct.	P.ct.	P.ct.	P.ct.
Moisture	5.50	4.80	5.24	6.15	——	——
Ash	0.23	0.52	0.78	1.00	0.85	1.67
Protein	9.63	5.07	10.09	16.42	19.72	7.52
$(NH_4)_2SO_4$	0.61	0.47	0.52	1.91	——	——
Pentosans	80.70	82.83	79.42	68.59	67.25	54.28
Uronic acids	none	none	none	none	——	——
Galactose	none	none	——	——	——	——
Fermentable sugars after hydrolysis	none	none	none	none	——	——

values for moisture, ash, and total nitrogen (Kjeldahl) were obtained by the usual methods. Any nitrogen contamination in the form of ammonium sulfate was determined by distilling samples in 0.3% sodium hydroxide for 20 minutes. Pentose sugars were determined by the phloroglucinol method and calculated as pentosans using Krober's conversion factors. Quantitative determinations of galactose were run according to Van der Haar (1920, p. 123). Hexose uronic acid determinations were carried out by two methods, both of which were in close agreement. The volumetric method described by Dickson, Otterson, and Link (1930) was used, and as a check, the gravimetric method described by Van der Haar (1920, p. 71). Five-tenths per cent carbon dioxide was obtained from all these gums, corresponding to about 2.5% hexose uronic acid. However, when the procedure was carried out on samples of the pure pentose sugars, xylose and arabinose, containing no uronic acids, the same amount of carbon dioxide was obtained. The samples were heated for 4 or 5 hours in boiling 12% hydrochloric acid in a stream of carbon dioxide free air. It seems reasonable to suppose a certain amount of oxidation takes place, with the production of a corresponding amount of carbon dioxide. Anderson (1931) refers to similar results.

It is apparent that the gums are composed principally of polymerized pentose sugars contaminated with a small amount of protein material that was not eliminated in the purification processes. The absence of hexose uronic acids is significant in that it distinguishes these gums from the hemicelluloses isolated by other workers using dilute sodium hydroxide. It is to be noted that the analytical data do not account for the difference in color and appearance of the different gums.

In Table I the nitrogen from the ammonium salts (calculated as ammonium sulfate) was subtracted from the total Kjeldahl nitrogen and the remainder was assumed to be protein nitrogen. This assumption was justified by the following experiments: Biuret test showed the presence of polypeptide linkages. A qualitative test for loosely bound sulfur as found in cystine was given by aqueous solutions of the gums. A quantitative determination of sulfur yielded only traces of sulfur. Van Slyke analyses for α-amino nitrogen on four of the gums in aqueous solution were so low as to be indicative of unhydrolyzed protein material. Two samples of gums were hydrolyzed for 3 hours in 4% sulfuric acid at 90° C. and then analyzed for free amino nitrogen. A slight hydrolysis of protein material might be expected under these conditions and the data in Table II indicates a small increase in free amino nitrogen. These data together with the method of isolation leave little doubt as to the presence of small amounts of protein.

Since pentosans were the major constituent, further investigation of this fraction seemed warranted. Qualitative tests showed no free reducing sugars until after hydrolysis by hot dilute mineral acids. To determine the proper conditions for hydrolysis the rate of hydrolysis in hot 4% sulfuric acid was determined as follows: exactly 1.8680 gms. of wheat gum—1927 was weighed into a 100 cc. volumetric flask. Twenty cubic centimeters of distilled water was added and the mixture

TABLE II

FREE AMINO NITROGEN IN THE CEREAL GUMS

Identification	Amino N in the gum	Amino N as % of total N
	P.ct.	
Wheat gum—1927	0.16	3.51
Durum gum—1929	0.13	5.20
Durum gum—1930	0.19	4.10
Rye gum—1930	0.32	3.90
Durum, 1930—hydrolyzed	0.17	9.26
Rye, 1930—hydrolyzed	0.46	13.38

was allowed to stand over night to effect uniform dispersion. Eighty cubic centimeters of 5% sulfuric acid was added and the solution was thoroughly mixed. A drop or two of 4% acid was sufficient to bring the solution to volume. Five cubic centimeter samples were pipetted into small glass vials. The tops of the vials were loosely adjusted, eliminating increase in pressure on heating that might increase the rate of hydrolysis. The vials were placed in an oven maintained at 80° C. At stated time intervals, vials were removed and their contents washed into a beaker with cold distilled water. Fehling's solution was added and the reducing sugars determined in the usual manner. These data are presented in Table III.

The gum is readily hydrolyzed under these conditions with no charring or other evidences of decomposition. The maximum yield of reducing sugars is reached in 2.5 hours, after which there is a slight decrease, which is probably due to the conversion of some of the pentose sugars to furfural.

Under the conditions noted above, the gums were hydrolyzed and attempts were made to identify the sugars which were present.

WHEAT GUM—1927. 7.1713 gms. of gum were dissolved in 400 cc. of 4% sulfuric acid and heated in a boiling water bath for 3 hours. The liquid was filtered and solid barium hydroxide was added until the hydrolysate was only faintly acid. The precipitated barium sulfate was removed by suction filtration and the filtrate was evaporated under reduced pressure to a few cubic centimeters of an amber sirup. The addition of 95% ethanol produced a small flocculent precipitate which

was removed by filtration. A precipitate at this point should be the barium salts of hexose uronic acids, if they were present, but in this case the precipitate appeared to be a protein residue. The alcoholic filtrate was again evaporated under reduced pressure to 4 cc. of an amber sirup. The sirup was seeded with a few crystals of l-arabinose and placed in an icebox for 2 weeks. When removed, a few cubic centimeters of 95% ethanol was stirred into the thick mass of crystals and sirup to facilitate filtration. The crystals were filtered, washed with

TABLE III

RATE OF FORMATION OF REDUCING SUGARS ON HYDROLYSIS OF WHEAT GUM BY 4% H_2SO_4 AT 80° C.

Sample number	Time of heating	Reducing sugars calculated as glucose
	Minutes	P.ct.
1	0	0.20
2	15	16.16
3	30	39.72
4	45	50.53
5	60	65.59
6	75	82.69
7	90	89.50
8	105	89.08
9	120	74.00
10	150	90.58
11	195	88.54
12	240	85.97
13	270	88.65
14	330	88.43
15	390	85.11
16	450	87.26
17	1410	85.97
18	2880	83.50
19	3120	77.51

a few cubic centimeters of ethanol and ether, and dried. The yield was about 0.7 gm. of a snow white powder. The phenyl osazone melted, after several recrystallizations from hot 60% ethanol, at 160° C. Both *l*-arabinosazone and *l*-xylosazone melt at 160° C. Bertrand's test for xylose was negative. $[\alpha]_D$ for the sugar was + 101.5°. For *l*-arabinose $[\alpha]_D$ = + 104°.

Using quantitative technique a sample of hydrolyzed gum was examined in the saccharimeter. On the basis of the analyzed content of pentose sugar $[\alpha]_D$ was calculated to be + 49.3°. The phenyl osazone prepared from this sirup melted sharply at 160° C. after several recrystallizations from hot water and when examined under the microscope appeared to be arabinosazone. Bertrand's test for xylose was again negative.

The evidence clearly indicates the presence of *l*-arabinose. The low optical rotation of the hydrolyzed sirup seems to show the presence

in appreciable amounts of another substance with a much lower rotation than arabinose. Although Bertrand's test was negative, it seems most likely that d-xylose is the substance responsible for the low optical activity of the hydrolyzed sirup. Apparently some impurity (protein residues?) prevented the crystallization of the cadmium xylonobromide in its characteristic form for this test failed even when xylose was added to the hydrolyzed sirups. The possibility still remains that the low optical activity of the sirup was due to some unsuspected non-reducing hexose polymer, although this seems unlikely.

DURUM GUM—1929. 2.6175 gms. were dissolved in 150 cc. of the acid and the sugar sirup was obtained in the usual manner. About 100 cc. of 95% ethanol were added producing a small white gelatinous precipitate, apparently a protein residue. The alcoholic filtrate was evaporated to 5 cc. of a wine red sirup and seeded with *l*-arabinose and placed in the icebox. About 0.197 gm. of a crystalline sugar was obtained, which was washed with ethanol and ether and dried. The phenyl osazone after repeated crystallizations, melted sharply at 160° C. Bertrand's test for xylose was negative.

1.5669 gms. of the gum were hydrolyzed and the sirup obtained in the usual manner, observing due precautions as to any loss of material. This was washed into a tared beaker. The total weight of the sirup was 22.5197 gms. The observed rotation of the sirup in a 200 mm. tube was + 16.5° V. $[\alpha]_D = + 40.8°$. Bertrand's test for xylose in the hydrolyzed sirup was negative and the phenyl osazone, after repeated crystallization, melted slowly with decomposition at 145° to 150° C. When examined under the microscope, however, the phenyl osazone appeared to contain many crystals similar to phenyl arabinosazone.

The sugar isolated was in all probability *l*-arabinose. The evidence for the presence of xylose was indirect and much the same as discussed under wheat gum—1927.

DURUM GUM—1930. 2.3008 gms. were dissolved in 150 cc. of 4% sulfuric acid, hydrolyzed and neutralized as described. The precipitated barium sulfate was dried and examined for reducing sugars and nitrogen by the Kjeldahl method. Less than 0.1 per cent reducing sugars, calculated as glucose, and only 0.12% nitrogen was found, thus indicating no appreciable loss of material in this operation. The filtrate was evaporated to a sirup, treated with 95% ethanol; and the alcoholic filtrate was again evaporated to a sirup. No crystals formed after seeding with *l*-arabinose and standing in icebox for 2 weeks. The phenyl osazone was not identified because of the extremely small yield.

Exactly 0.6391 gm. of gum was hydrolyzed, neutralized and evaporated to a sirup containing 10.0158 gms. of water. The observed optical rotation of this sirup in a 200 mm. tube was + 7.45° V. $[\alpha]_D$ = + 21.5°. For d-xylose $[\alpha]_D$ = + 19.8°.

The phenyl osazone melted sharply at 159° C. and under the microscope appeared to be crystals typical of phenyl xylosazone but Bertrand's test for xylose failed.

The evidence indicates that this gum is composed entirely of xylose, differing from the others. It might be noted that this gum differing in appearance and texture from the other gums answers to the description of the xylan isolated by Link (1930) from corn seedlings.

RYE GUM—1930. Since only a small amount of this gum was available and contained appreciable amounts of impurities, investigation of the hydrolytic products was restricted to the preparation of the phenyl osazone, which melted sharply at 160° C. after repeated crystallizations. Characteristic crystals of phenyl arabinosazone were observed under the microscope.

Only small amounts of wheat gum—1931 and the barley gum were prepared, hence their hydrolytic products could not be determined. Further investigation of these gums as well as of the rye gum is contemplated.

The Gums as Lyophilic Colloids. As has already been noted, the cereal gums disperse in water to form extremely viscous sols. Accordingly an attempt was made to measure the degree of hydration of the dispersed gum by using viscometric technic.

2.2646 gms. of wheat gum—1927 were dispersed in 30 cc. of water and thoroughly mixed. The viscosity was determined in an Ostwald viscometer. The sol was then diluted with 10 cc. of water and the viscosity again determined. This procedure was repeated several times. The data together with a number of calculated ratios are shown in Table IV. The relative viscosity (η_r), which is the ratio $\frac{t_{(sol)}}{t_{(solvent)}}$ when plotted against concentration yields a curve, showing that viscosity increases more than linearly with concentration. The form of the curve suggested a Freundlich adsorption isotherm; accordingly the ratio $\frac{\log (t - t_0)}{\log C}$ was calculated. This ratio should be a constant if the adsorption formula was strictly followed. The fourth column of Table IV shows that the ratio increases with increasing concentration. The specific viscosity (η_{sp}) which is the ratio $\frac{t - t_0}{t_0}$, likewise increases more than linearly with concentration as is shown by the ratio $\frac{\eta_{sp}}{C}$. Heidel-

Berger (1932) found the ratio $\frac{\eta_{sp}}{\sqrt{C}}$ to be approximately a constant over a limited range of concentration in the case of certain of the "immunologically specific polysaccharides" but this ratio was far from constant for our gum sols.

Kunitz (1926) proposed the formula $\eta_r = \frac{1 + 0.5\,\phi}{(1 - \phi)^4}$ as an improvement of the Einstein lyophilic colloid equation. In this formula ϕ is the per cent of the total volume of the system occupied by the disperse phase. Accordingly, the ratio ϕ/C expresses the volume in cubic centimeters which is occupied in the sol by one gram of the solute. From Table IV it will be noted that this ratio approximates a constant,

TABLE IV

THE DEGREE OF HYDRATION OF WHEAT GUM—1927 AS DETERMINED BY VISCOMETRIC TECHNIC

Gum conc.	Time of outflow	$t-t_0$	$\frac{\log t-t_0}{\log C}$	η_r	η_{sp}	$\frac{\eta_{sp}}{C}$	$\frac{\eta_{sp}}{\sqrt{C}}$	ϕ	$\frac{\phi}{C}$
Gms.	Seconds	Seconds						P.ct.	Cc. per gram solute
7.33	323	313	0.347	32.3	31.3	4.27	11.56	55.45	7.6
5.36	137	127	0.347	13.7	12.7	2.37	5.49	45.33	8.5
4.33	83	73	0.342	8.3	7.3	1.69	3.50	38.30	8.9
3.64	60	50	0.330	6.0	5.0	1.37	2.62	33.40	9.2
3.13	42	32	0.329	4.2	3.2	1.02	1.81	27.80	8.9
2.77	37	27	0.309	3.7	2.7	0.97	1.62	25.58	9.2
2.45	31	21	0.294	3.1	2.1	0.86	1.34	22.50	9.2
2.21	27	17	0.280	2.7	1.7	0.77	1.14	20.05	9.1
2.01	25	15	0.258	2.5	1.5	0.75	1.06	18.75	9.3
0	10	0							

and that each gram of solute occupies about 9 cc. of volume when in the sol state, although there appears to be some decrease in hydration of the micelles after the disperse phase occupies about 40% of the total volume. We must accordingly conclude that these gums are typical hydrophilic colloids, characterized by a high degree of hydration, which accounts for the extreme viscosity of their sols.

Examination of Gums Prepared by R. K. Larmour (1927). A series of gums were obtained by Larmour (1927) by the alkali extraction of a number of cereal grains. These samples being available, a few experiments were undertaken to ascertain whether or not they were identical with the gums which we had been studying. They yielded only about 3.15% pentosans, nitrogen was less than 1.0%, and ash was 3.0 to 4.0%. When hydrolyzed in hot 4% sulfuric acid for several hours, a sirup was obtained that was readily fermented by yeast. This sirup yielded

glucose phenylosazone. These experiments were sufficient to demonstrate that these gums were of an entirely different nature from the pentosan gums which we are reporting. Probably they are similar to the hemicelluloses reported by Ritthausen (1867), Effront (1897), and Clayson and Schryver (1923).

Discussion

The low yields are especially noteworthy. Variations of the method described by Hoffman and Gortner (1927) apparently simplified the method but failed to materially increase the yields. Bailey (1925) indicates as much as 3% pentosans in clear wheat flours. It seems likely from these studies that a large part of this pentose material is not extracted by neutral salt solutions. Possibly the total pentosans in wheat flour are largely the type of gum (or hemicellulose) described by Norris and Preece (1930) and are extracted only by dilute alkali. Variations in the procedure yielded gums of decidedly different appearance. These differences were not revealed by the analytical methods employed. Probably small amounts of impurities, variations in moisture, or types of pentose sugars are the factors responsible for these differences. The identification of the pentose sugars was hampered by the small quantities of gums which could be isolated. As a result the evidence is not conclusive. Arabinose was positively identified in several of the gums but the presense of xylose could not be determined with certainty. A consideration of the evidence presented, however, seems to indicate that the gums are largely mixed polymers of xylose and arabinose with the exception of durum gum—1930 which appears to be a polymer of xylose alone. This particular sample differed in appearance from the other gums and answered the description of the xylan prepared by Link (1929, 1930).

Conclusions

1. Gums similar to the wheat gum described by Hoffman and Gortner can be prepared from durum, rye, and barley flours. This method has been simplified by extracting the flour with half saturated ammonium sulfate solution.

2. The gums contain no hexoses or hexose uronic acids.

3. Analysis indicates that the gums are largely pentose polymers admixed with some protein material.

4. The rate of hydrolysis in hot dilute sulfuric acid is typical of pentose-polymers of this type.

5. Evidence indicates that the pentose sugars formed by hydrolysis of the gums are mixtures of xylose and arabinose except in durum gum—1930 which seems to be a pure xylan.

6. The nitrogen present is in the form of protein, probably adsorbed or closely associated with the gum.

7. The gums differ from the hemicelluloses prepared by Larmour (1927) which contain large quantities of hexose sugars and at the most small quantities of pentose sugars.

8. These pentose gums form typical colloidal sols with highly hydrated micelles. Viscometric data indicate that each gram of the gum in the sol state occupies about 9 cc. volume, accordingly in the sol state the gum in hydrated to the extent of at least 800%.

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PHOTOCHEMICAL ACTION, A CAUSE OF RANCIDITY [1]

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(Read at the Convention, May, 1932)

Rancidity in foods is generally assumed to be caused by oxidation or hydrolysis aided by one or more of several factors, such as light, moisture, heat, metallic catalysts, or enzymes. Experiments conducted by the Food Research Division of the Bureau of Chemistry and Soils on the role played by light in the production of rancidity have shown that the action of light in this respect varies with the wave-lengths of the light. Some wave-lengths promote rancidity while others are inert to this form of spoilage. These experiments have been carried on along the following lines upon a number of foods and feeds, including rice bran and polish, butter, lard, salad oils, mayonnaise, potato chips, biscuits, and crackers:

(*a*) The effect of ultra-violet light.
(*b*) The effect of sunlight.
(*c*) The effect of various colored cellophane and paper wrappers.
(*d*) The effect of monochromatic light.
(*e*) The effect of aerating food products wrapped in (1) black, (2) green, and (3) white paper.
(*f*) The effect of excessive moisture in samples (1) wrapped in black, and (2) unwrapped.

In many of our experiments color filters of definite and known light transmission were employed. These were from the Corning Glass Works, Corning, New York. In all cases the samples were tested for rancidity organoleptically, by the sense of smell, and also with the von Fellenberg modification of the Schiff fuchsin reagent or according to the Kreis procedure. In many cases the fat or oil extracted from the samples was analyzed for the iodine number, acid number, and saponification number, as well as the peroxide value. The tota acidity of the samples was also determined.

No well defined correlation was noted between the values obtained for these so-called "fat constants" and rancidity, except possibly in the case of the peroxide number which is affected by the action of light and which in turn is correlated with rancidity.

[1] Food Research Division Contribution No. 151.

Preliminary Experiments

A distinct test for rancidity was obtained both organoleptically and with the von Fellenberg reagent on rice polish exposed to the direct action of the sun for several days. Similar results were obtained after rice polish had been exposed to the action of ultra-violet light for several hours.

Rice bran and polish were wrapped in various colored cellophane and exposed to direct sunlight. This experiment resulted in the discovery which is the subject of this paper, viz., that certain wave-lengths of light promote rancidity, while certain others exert a protective action against this change.

In every case where the samples were wrapped in a certain shade of green the appearance of rancidity was delayed for an appreciable length of time, while those in wrappers of other colors gave, in a relatively short time, a positive test for rancidity.

Wrapping the products with black resulted in the retardation of rancidity due to the fact that black absorbs all wave-lengths, thus permitting none to act upon the food material.

The Effect of Monochromatic Light

Through the cooperation of the Smithsonian Institution it was possible to study the effect of various monochromatic wave-lengths upon the production of rancidity in foods. Butter was exposed for 17 hours to the action of light rays produced by a monochromator having a range of from 3020 to 5461 Ångström units. A test of the butter thus subjected to various wave-lengths of light showed a positive reaction for rancidity with the modified Schiff's reagent in every case except where the butter had been acted upon by green light at a range of approximately 5461 Ångström units.

The experiments were repeated with glass color filters. Foods such as butter, lard and oils, which, under certain conditions, readily become rancid, were placed under these filters and exposed to ordinary daylight. Here again it was shown that covering with a green wrapper, which removed practically all radiation except that in the range of 4900 to 5600 Ångström units, exerts a protective action, retarding the appearance of rancidity through the removal of active radiation; whereas, the products under the other color filters gave an organoleptic test as well as a von Fellenberg test for rancidity.

The Effect of Oxidation

That oxidation in the absence of light does not hasten rancidity is shown by the following simple experiment. Fresh rice bran was placed in two bottles, one wrapped with black paper and the other unwrapped. Air was circulated through each of these samples for ten days. At the

end of that time the bran kept in the unwrapped bottle gave a positive test for rancidity, both organoleptically and with the von Fellenberg reagent. The bran in the black-warpped bottle gave negative tests.

The Effect of Metallic Catalysts

In another experiment air was passed for several days through samples of bran to which finely divided copper and iron filings had been added to serve as catalysts. Here again the samples of bran which were protected with a black wrapper remained sound and failed to give tests for rancidity.

The Effect of Excluding Air

A sample of rice bran was placed in two bottles, one protected with a black wrapper, the other unwrapped. From each the air was displaced with carbon dioxide by means of dry ice and the bottles were closed for several months. At the end of that time, the unwrapped sample gave a positive test and the one wrapped in black a negative test for rancidity.

Rice bran press cakes were dipped in gelatin and allowed to dry. Several of these gelatin-coated cakes were wrapped with black paper; others were kept unwrapped. Both sets were exposed to ordinary sunlight for several weeks, after which it was noted that the cakes wrapped in black were still sound and free from rancidity, while the unwrapped ones gave positive tests both organoleptically and with the fuchsin reagent.

The Effect of Excessive Moisture

A sample of rice bran containing 17% H_2O was placed in bottles, one wrapped in black and the other unwrapped. After exposure to ordinary light for several months the unwrapped sample was rancid, while that in the black wrapper was still sound.

Potato chips, freshly made by the U. S. Bureau of Home Economics, were placed in two packages, one the ordinary package and the other a specially prepared package of the proper shade of green. The oil used in the preparation of these chips was also placed in containers, one wrapped with black paper and the other container unwrapped. The chips contained in the ordinary paper bag were rancid at the end of two weeks, whereas those in the green bag were free from this form of spoilage.

The samples of oil were tested at weekly intervals for the peroxide value by Wheeler's modification (Oil and Soap, April, 1932, p. 89) of the Lea method. The oil which had been kept in the unwrapped bottle gave the following peroxide values in five weekly tests: 8, 49, 74, 101 and 207, whereas that in the bottle wrapped with black gave 8, 17, 29, 31 and 36, respectively. The oil in the clear or unwrapped

bottle thus underwent chemical changes at a tremendously faster rate than that in the protected bottle.

Supplementing these experiments, Dr. L. B. Kilgore [2] has studied the effect of passing oxygen and air through cottonseed oil under the following conditions: 1, Control; 2, containing 0.05% hydroquinone; 3, in a bottle wrapped with black paper; 4, in a bottle wrapped with white paper.

The initial peroxide value was less than one. At the end of 33 days the peroxide values were as follows: (1) 62, (2) 8, (3) 22, and (4) 30, thus indicating that the "control" changed rapidly due to the effect of light, whereas the oil protected from light or treated with hydroquinone underwent much slower oxidation. While a black wrapper is apparently not so effective as the presence of an antioxidant, still it must be remembered that such products as hydroquinone, pyrogallol, Beta naphthol, and guiacol are not substances fit to be incorporated in food products.

The use of a black wrapper or one of the proper shade of green will protect the food product for a sufficiently long time to permit of its sale and consumption before spoilage by rancidity develops. This has been amply proven by laboratory tests of commercial samples of biscuits and crackers, pretzels, potato chips, etc., submitted to us by the manufacturers. Samples of these products were wrapped with black paper, some with an extra layer of white paper, and in some cases with green material. When certain crackers were wrapped with black and also with white paper and kept for nine months, those in black were still fresh and edible, while those in white were rancid and absolutely inedible. Potato chips wrapped in green were still edible after one month, while those in the original bag were rancid within two weeks. Pretzels were tested for the first time eleven months after being wrapped. Those wrapped in black were still good, while those in white were rancid and apparently had been inedible for some time.

Summary

1. The exclusion of light retards the development of rancidity.

2. Green light (delimited with 4900 to 5600 Ångström units) is the main portion of the visible spectrum which is inactive with regard to the development of rancidity.

3. Selective ultra-violet light hastens the development of rancidity as compared with natural sunlight.

4. Black or green containers are a practical and effective means of protecting from spoilage food materials subject to certain types of rancidity.

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THE EFFECT OF TEMPERATURE AND OF THE INCLUSION OF DRY SKIMMILK UPON THE PROPERTIES OF DOUGHS AS MEASURED WITH THE FARINOGRAPH [1]

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(Read at the Convention, May, 1932)

The introduction of the farinograph early in 1931 immediately created interest in the possibilities of certain mechanical tests of dough which could be effected by means of this device. We were afforded an opportunity to use the farinograph during recent months. The form in which it was delivered to us is essentially similar to the descriptions appearing in German and American advertising literature of 1931. The two blades of the small dough mixer rotate once in 1.87 and 1.25 seconds, respectively. The wall of the mixer is of solid brass about 5 mm. thick and has no water jacket. Relative plasticity of the dough is registered and recorded in terms of the force applied in rotating the mixing blades through the dough at constant speed. Doughs which are *high in plasticity* or relatively *stiff* require the application of more force to the mixing blades than slacker, more mobile doughs. Measurements in farinograph degrees tend to fall, therefore, as doughs become slacker and more mobile. Bread doughs of normal consistency register about 500 units on the plasticity scale of the farinograph when freshly mixed.

Early in these studies it developed that a dough which was at room temperature after the first 4 or 5 minutes of mixing would increase in temperature through five or six degrees Centigrade before the mixing period of 27 minutes was concluded. At least this was the case when the maximum plasticity was adjusted to the equivalent of 500 units.

It was also observed that certain dough ingredients markedly affected the amount of mixing required to reach the maximum plasticity even when using the same flour. This in turn resulted in varying dough temperatures at the time these maxima were reached. Since dough absorption capacity is presumed to be determined by the quantity of water required to exactly attain this fixed plasticity, it

[1] Paper No. 1102, Journal Series, Minnesota Agricultural Experiment Station.
[2] American Dry Milk Institute Fellow.

becomes necessary to so adjust conditions that no error is introduced in determining dough consistency at its highest point.

It seemed inevitable that temperature differences, as here observed, would have an appreciable effect upon the recorded plasticity values. A dough with a maximum reading after 16 minutes of mixing would be several degrees warmer than another dough prepared with the same flour whose maximum might come after only 6 minutes. Consequently, the adjusted absorption might be in error.

It is well known that the relative mobility of many viscous and plastic materials is a function of temperature, but no data were available which disclosed the specific effect of temperature upon the plasticity of doughs. A series of observations were made, therefore, to determine the thermal coefficient of dough plasticity. A mixture of water with a soft wheat flour at an observed temperature was made in the farinograph. The maximum plasticity was reached in about 4 minutes. Mixing was then discontinued and the dough temperature was noted. After cleaning the mixing bowl, new dough ingredients were introduced in the same proportion but at a different temperature and the mixing curve recorded on the same graph. Six such doughs were mixed in the first series with dough temperatures as the variable and ranging from 21° to 26½° C., and a maximum plasticity range of 510 to 670 units. The magnitude of plasticity in farinograph units decreased regularly with temperature, that is, the warmer doughs were more mobile, as shown in Figure 1.

In another series of observations, water absorption was made a variable as well as temperature. On increasing the percentage of water, the maximum plasticity reached by the dough during the mixing period was lowered in terms of farinograph units. These results are presented in Figure 2. In this series these maxima were in the range of 340 to 500 units. This range was not covered in sufficiently definite increments nor were the tests replicated enough to afford an accurate estimate of the effect of temperature at all of the plasticity levels when the latter was varied by adjustments of the percentage of water. In general, a slack dough (340 ± units) would decrease in plasticity by about 12 ± units when the temperature was increased through one degree Centigrade. Stiff doughs (670 ± units), however, would decrease about 40 ± units on the same increase in temperature. Moreover, the stiff doughs register a larger increase in temperature per unit of time in mixing than slack doughs, which further complicates the application of corrections for temperature changes.

A simple procedure has been followed in our laboratories to overcome these decided temperature increases when working with doughs of a 500 unit maximum plasticity. By experiment, it was determined

that if dough ingredient temperatures were so selected that the dough when formed attained a temperature of 29° to 30° C., the heat generated during the mixing process would be dissipated when working in room temperatures of 21° to 22° C.

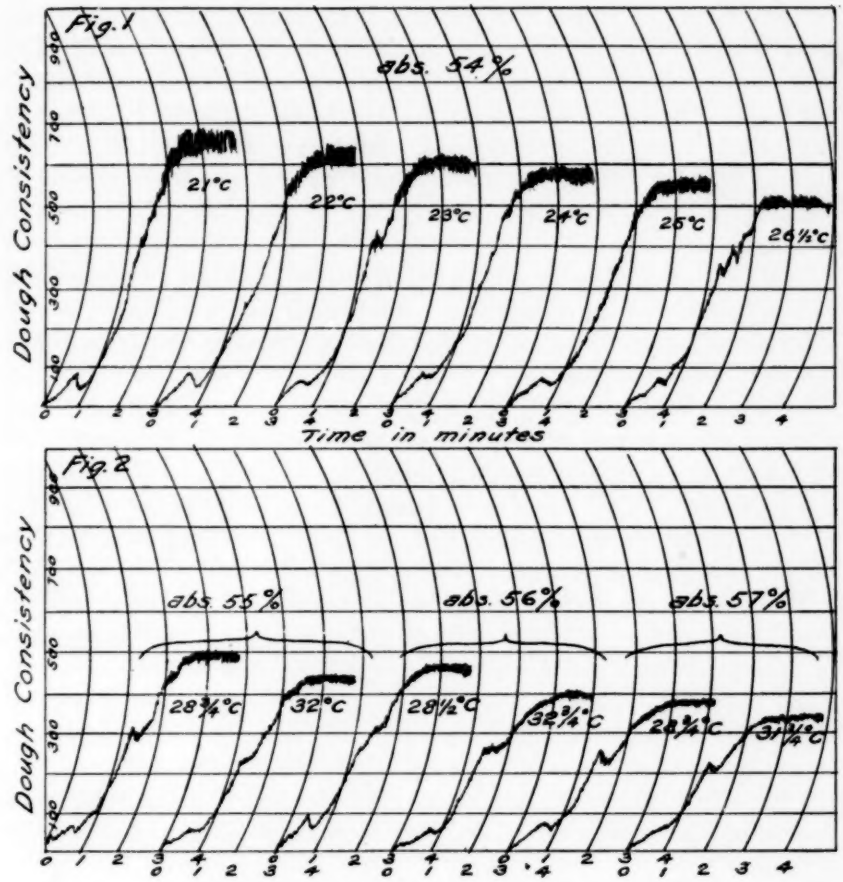

Fig. 1. The effect of temperature upon plasticity readings in stiff doughs.
Fig. 2. The effect of temperature upon plasticity readings in doughs mixed at varying levels of absorption.

In preparing doughs designed to have a particular level of temperature when first mixed, it is necessary to not only take cognizance of the temperature of the mixing machine and of the several dough ingredients, but the moisture content of the flour must be considered as well. As was shown by Winkler and Geddes [3] the heat of hydration of flour is a function of moisture content, and with abnormally dry flours

[3] Winkler, C. A., and Geddes, W. F. The heat of hydration of wheat flour and certain starches including wheat, rice, and potato. Cereal Chemistry **8**: 455–475 (1931).

an allowance must be made for the heat energy released when water is added in mixing the dough.

When the doughs were mixed so that their temperature was about 29½ ± ° C., it was possible to obtain good agreement in replicate curves. This control has been of special value in comparing absorptions in doughs of different types since temperatures have rarely varied more than 0.5° during the entire mixing period of 27 minutes or more. It is realized that during periods of warm weather, this method could not be used without either resorting to cooling of the air or to mixing the doughs at higher temperatures. In the event that water-jacketed cooling systems are later made available by the manufacturers of the farinograph, the procedure outlined can be regarded as a temporary measure.

Our investigations have been primarily concerned with measures of baking quality in dry skimmilks. Since flour and water are the only ingredients commonly used in studying doughs with the farinograph, the first determinations were made in comparing such simple doughs with those containing 6% of dry skimmilk as an added ingredient. From the first it was apparent that the usual full effectiveness of dry skimmilk in increasing dough absorption was not disclosed in such comparisons. Other methods of manipulation, such as reconstitution of the milk for an hour previous to use and preliminary mixing of the dough in high speed machines, did not aid materially.

Since the mechanics of the method did not appear to be at fault, our attention was next directed toward the formula used. Measurements of absorption with the farinograph in milk-free and milk doughs when using the usual baking formula gave the customary increases due to dry skimmilk. Dough ingredients were then investigated separately, and it was found that it was only necessary to include salt (NaCl) to effect the increase in absorption, normal to milk doughs. This ingredient was accordingly incorporated in certain of the doughs used in studies of dry skimmilk.

When thus used in the proportion of 1.75%, in a series of comparisons involving 3 flours and 11 different milk powder samples, the average increase in absorption due to 6% dry skimmilk was about twice the magnitude of that when salt was not included. In addition to the effect upon the maximum plasticity attained by the milk doughs, the salt also had a pronounced effect upon the rate of dough formation. With the use of salt in milk doughs, the mixing time requirement to obtain essentially the maximum plasticity was from 2 to 4 times as long as in milk-free doughs, depending upon the type of flour and milk. When salt was not used, milk-free and milk doughs apparently formed at about the same rate, although the latter were less reduced in

plasticity with extended mixing than the former. Apparently, there is a pronounced effect of salt upon certain milk components when placed in a dough which is reflected in its rate of hydration as well as in its total hydration capacity.

Six samples of dry milk were subjected to baking tests in two laboratories and were also used in making duplicate farinograph determinations with two flours, both with and without salt. By using the amount of decrease in plasticity from the maximum due to extended mixing of salt-containing doughs as a measure of dough quality, it was found that the rating of the milk samples agreed very well with scores from baking tests. This rating of the milks was identical when used with either of the two flours, but the rating was quite different when comparing doughs of the same flour, with and without salt. The poorer baking quality milks are apparently affected in a different way by the salt addition than those of superior characteristics as reflected by the rate of hydration of the various milk doughs and by the dough resistance to extended mixing. This suggests a field for further work in using ion or salt effects as a means of equalizing and improving dry skimmilk quality.

Duplicate farinograph determinations were made with 14 dry skimmilk samples in doughs containing 1¾% of salt. The agreement between duplicated tests was fairly good. Baking quality scores had been obtained by the producers of this milk after replicate bakings which in some cases involved checks in more than one laboratory. When the correlation coefficient is calculated for these baking scores and the amount of fall of the doughs from maximum plasticity in the farinograph, a value of $r = -.76$ is obtained. According to Fisher's [4] tables, this coefficient indicates in this case that odds are greater than 100 to 1 that this agreement is not due to chance.

In 1931, Skovholt and Bailey [5] reported certain results obtained in a study of the effects of heat treatment of fluid milks when tested as such, as well as the results of preheating of fluid milk upon the dried product. Because of observed differences in farinograph curves with doughs containing different dry skimmilks, it seemed that interesting information might be secured through the application of similar tests to doughs made with fluid milks that had been subjected to controlled heat treatments.

Fresh skimmilk was secured from the University creamery, divided into five aliquots, and four of the portions subjected to heat treatments for 30 minutes at 63°, 73°, 83° and 93° C., respectively. Plasticity determinations were then made with the farinograph upon doughs

[4] Fisher, R. A. Statistical methods for research workers. 3d edition. London, 1930.
[5] Skovholt, Oscar, and C. H. Bailey. Cereal Chem. 8: 374–380 (1931).

containing each of these milks and also with a milk-free dough. The milk was used in a quantity sufficient to supply all needed liquid to make doughs of proper consistency. Two series of doughs were made, one salt-free and the other containing 1.75% common salt. The entire experiment was then repeated with a fresh lot of skimmilk.

Resulting plasticity curves differed as a result of the heat treatments both in the rate of dough formation and in the apparent dough resistance to mixing. The latter quality was measured by determining the amount of fall of the curve after passing through the maximum plasticity. For convenience, the rate of decrease in plasticity is measured by adding the departures from the maximum at 2 minute intervals for a unit period of time. Thus, with a flat curve indicating no change in plasticity, the departure from the maximum would be recorded as zero. The summation of these decreases in plasticity are recorded for each dough in Table I. In addition, the relative vis-

TABLE I

VISCOSITIES OF MILKS AND FALL IN PLASTICITY OF DOUGHS CONTAINING THEM CAUSED BY EXTENDED MIXING

	Water	Unheated milk	Milk heated 63° C.	Milk heated 73° C.	Milk heated 83° C.	Milk heated 93° C.
Viscosity sec/5	138	199	198	208	215	220
Doughs without salt						
Summation of plasticity decrease	515	280	275	50	230	170
Doughs containing 1.75% salt						
Summation of plasticity decrease	135	1525	1270	165	75	30

cosities of the milks subjected to various heat treatments, in terms of the time of outflow from an Ostwald viscosimeter, are recorded in the same table.

Absorptions were the same in the milk doughs of each series and the peaks of all plasticity curves were practically at the same level. This suggests that reported differences in absorption caused by different milk samples are not primarily due to a difference in maximum hydration capacity, but are due to the inability of certain milks to retain or cause the dough to retain this water in close combination for more than a short time when subjected to mechanical treatment. Under practical conditions the absorptions of doughs containing these milks would probably be adjusted at various levels due to inability to exactly attain the plasticity peak in mixing and also due to the

probability of greater slackening during fermentation with doughs containing the milks of poorer quality.

The milks were all nearly the same in effect on absorption capacity when adjusting plasticity to identical levels. However, it will be observed that the doughs containing unheated or lightly heated milk rapidly decreased in plasticity after passing through the maximum. The difference in absorption between milk and water doughs was about 3½% greater when salt was present than in the salt-free doughs. Since the maximum hydration capacity of the milks was not reached in salt-free doughs, the slackening with mixing was lessened, was not greatly different with any of the milk samples, and was considerably under that of the water dough. In the series of doughs containing salt, the ability of doughs to retain their original maximum level of plasticity was a function of the temperature to which the milk had been heated. (See last horizontal line of data in Table I.)

Summary

1. Temperature differences were found to cause plasticity variations in doughs of 12 to 40 farinograph units per degree Centigrade depending upon the stiffness of the dough. The comparison involved doughs ranging from 340–670 farinograph units in maximum plasticity.

2. Certain dough ingredients make additional mixing time necessary to reach the maximum plasticity which would lead to erroneous absorption data if dough temperatures were allowed to rise during the mixing process.

3. By using dough ingredients at a temperature several degrees above that of the room, it was possible to avoid temperature increases during mixing due to heat dissipation into the air.

4. Common salt was found to more than double the amount of additional water which could be added to reach a definite, maximum plasticity when using dry skimmilk.

5. The amount of decrease in dough plasticity with extended mixing was found to give a significant correlation with baking quality when studying a series of 14 dry skimmilks.

6. A good agreement was found in the rating of six of these milk powders by their plasticity curves when using two widely different flours in doughs containing salt.

7. Different ratings of the milks were obtained when using salt-free and salt-containing doughs.

8. Doughs containing heated fluid milks offer a resistance to plasticity decrease during mixing which is a function of the temperature to which the milks had been subjected, providing salt is a dough ingredient.

9. Absorption increases caused by milks are nearly the same regardless of milk quality if the highest plasticity level attained is used as a measure.

10. Significant differences in absorption are evident if consideration is given to the rapidity of plasticity decrease immediately after reaching the maximum when testing doughs containing poor quality milks.

VARIATION IN THE WEIGHT OF A GIVEN VOLUME OF DIFFERENT FLOURS—II. THE RESULT OF THE USE OF DIFFERENT WHEATS

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(Received for publication March 16, 1932) [1]

A number of investigators have made a study of specific gravity of wheat; Church (1867a, 1867b) found that corneous kernels contained more protein than starchy ones, and also that the corneous ones had a greater density. These observations have since been confirmed by Nowacki (1870), Wollney (1886), Snyder (1905), Roberts and Freeman (1908), Headden (1915), Bailey (1916), and others.

Nobbe (1876) found an increase in specific gravity of wheat with decrease in moisture content. Sharp (1927) regards moisture content, protein content, and protein quality as factors which exert a marked influence upon the density of wheat. Shollenberger and Coleman (1926) found the specific gravity of dark red spring wheat to be 1.3906, and the specific gravity of hard dark red winter wheat to be 1.4049.

Results given in a previous paper (Grewe, 1932) show considerable variation in the weight of a given volume of flour milled in the same section of the United States. Seven samples of flour milled in the State of Indiana in 1926 weighed as follows: 94.0, 91.2, 93.6, 92.6, 88.2, 92.5, and 87.7 grams per cup. Such variations in weight per given volume of flour milled in the same state were not unusual among the 323 samples of commercially milled flours. Flours milled in different sections of the country, such as Maryland and Missouri, were found to vary 18.8 grams in weight per cup.

Flour is the basic ingredient in batters and doughs and such variations in quantities taken, as those mentioned, would have a marked effect on the character of the baked product, and undoubtedly is an important cause of failure in baking. On account of the importance of the ratio of quantity of flour taken to other ingredients in baking, a study was made of some of the causes of variation in weight per given volume due to the wheat used in making the flour.

Experimental

Methods Used. The method for handling the flour was the same as that used in the previous study, except that a weighed quantity of

[1] Read at the Convention, May, 1930.

flour, 150 grams, was used. The factors taken into consideration in this investigation were as follows: Variety of wheat, protein content, wheat grown on irrigated and dry land, growing season, and locality.

Influence of Various Factors on Volume Weight

Variety of Wheat. Tests were made on this portion of the problem using 12 samples of flour milled from 2 varieties of wheat—American Banner and Berkeley Rock—furnished by the Agricultural Experiment Station of Indiana. Both varieties of wheat had been grown on 6 different types of soil. These 12 samples of flour were tested for weight per given volume. When grown on each of the 6 different types of soil, flour made from Berkeley Rock wheat weighed more per cup than flour made from American Banner wheat as may be seen in Table I. The 6 samples made from American Banner averaged 87.4 grams per cup. The 6 samples made from Berkeley Rock averaged 103.3 grams per cup. These results were confirmed by means of a second study with two other varieties, data for which are not included in the report.

TABLE I

EFFECT OF THE VARIETY OF THE WHEAT USED IN MAKING THE FLOUR ON THE WEIGHT OF A CUP OF FLOUR

Variety	Soil type	Crude protein (flour)	Weight per cup of flour
		P.ct.	Gms.
American Banner	Wauseon loamy fine sand	7.0	91.6
Berkeley Rock	Wauseon loamy fine sand	7.6	105.5
American Banner	Brookston clay	7.5	88.9
Berkeley Rock	Brookston clay	9.1	98.8
American Banner	Wooster silt loam	8.4	91.2
Berkeley Rock	Wooster silt loam	9.9	104.9
American Banner	Russell silt loam	7.7	83.2
Berkeley Rock	Russell silt loam	8.2	103.8
American Banner	Hillsdale sandy loam	9.8	84.6
Berkeley Rock	Hillsdale sandy loam	10.9	103.7
American Banner	Fox sandy loam	8.9	84.6
Berkeley Rock	Fox sandy loam	9.2	103.2

Protein Content of Flour. Protein determinations were made on many of the samples of soft wheat flour used in this investigation. A direct relationship between weight per given volume of soft wheat flour and protein content was not found. It is probable that other factors such as variety of wheat, granulation, etc., tend to mask the effect of protein content on the weight of the flour. Attention is called to the established and well known fact that hard wheat flour is higher in protein content and heavier in weight per given volume than soft wheat flour.

Flour from Irrigated and Dry Land Wheats. Experiments were

made with 20 samples of flour which were milled from 10 varieties of wheat furnished by the Bureau of Plant Industry, United States Department of Agriculture. One sample of each variety was grown on dry soil and the other on irrigated soil. These 20 samples of flour were tested for weight per given volume and the results recorded in Table II. The average weight per cup of the 10 samples of flour made from wheat grown on dry land was 102.9 grams. The average weight per cup of the flour made from wheat grown on irrigated land was 105.7 grams although it had been anticipated that flour made from wheat grown on dry land would weigh more per given measure than that grown on irrigated soil.

TABLE II

EFFECT ON THE WEIGHT PER GIVEN MEASURE OF FLOUR MADE BY GROWING WHEAT ON DRY AND IRRIGATED SOIL

Wheat	Soil	Moisture	Crude protein	Weight per given measure of flour
		P.ct.	P.ct.	Gms.
Marquis	Dry	13.25	15.82	103.4
	Irrigated	13.13	12.93	103.6
Marquello	Dry	12.80	15.92	101.7
	Irrigated	13.05	12.32	106.7
Ceres	Dry	12.73	16.31	104.1
	Irrigated	13.39	13.82	107.2
Reliance	Dry	12.53	16.28	97.5
	Irrigated	14.07	13.08	102.0
Reward	Dry	13.73	16.26	102.6
	Irrigated	13.90	15.18	103.9
Supreme	Dry	14.11	15.22	105.1
	Irrigated	14.16	11.55	108.1
Hope	Dry	13.50	15.96	103.9
	Irrigated	14.07	12.91	104.2
Pringles Champion	Dry	13.65	18.11	98.6
	Irrigated	14.08	13.51	101.7
Kubanka	Dry	12.41	16.86	107.6
	Irrigated	13.29	11.69	110.0
Mindum	Dry	12.29	17.50	105.0
	Irrigated	13.12	11.58	110.0

Growing Season. The average weight per given volume of flour made from wheat grown in 1926, 1927, 1928, and 1929, is shown in Table I of a previous paper (1932). It appears that in normal years there is not much difference in weight due to seasonal variation.

Locality in Which Wheat is Grown. The results presented in Table I of a previous paper (1932) indicate that the locality where the wheat is grown has as important an effect on the weight of the flour per given volume as it has on other well known factors, such as protein, baking strength, etc.

Summary and Conclusions

The variety of wheat from which the flour was made was found to be an important factor in weight per given volume of the flour.

Dry and irrigated soils produce wheats which mill into flour that will differ in weight per given volume. Flour made from the same variety of wheat was found to weigh more per given volume when grown on irrigated soil than when grown on dry soil.

Growing season is probably of minor importance in normal years. Flours made from wheat grown in the same locality for 4 different years were tested and found to be much the same in weight per given volume for each of the 4 years.

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